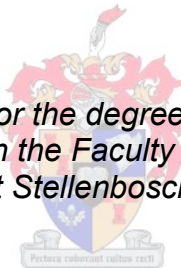


The effect of caffeine supplementation on Olympic-distance triathletes and triathlon performance in the Western Cape, South Africa

A double-blind, randomized, cross-over, controlled, clinical field trial exploring the performance benefits and factors influencing the ergogenic effects of caffeine supplementation in triathlon

by
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DECLARATION OF AUTHENTICITY

By submitting this dissertation electronically, I declare that the entirety of the work contained therein is my own, original work, that I am the sole author thereof, that reproduction and publication thereof by Stellenbosch University will not infringe any third party rights and that I have not previously in its entirety or in part submitted this dissertation for obtaining any qualification.

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Date: 15 October 2012

ABSTRACT

Background: Abundant evidence supporting the ergogenic effect of caffeine during endurance exercise exists. Single sporting events, laboratory based studies and inappropriate research design questions the applicability of these studies to triathlon performance.

Objectives: The main aims of this study were to i) investigate the ergogenic effect of caffeine supplementation during a triathlon; ii) evaluate parameters that could in part explain why caffeine supplementation is ergogenic, iii) investigate possible factors influencing the ergogenicity of caffeine supplementation and iv) investigate possible confounding factors influencing triathlon performance.

Methods: A double-blind, randomized, crossover, controlled, clinical field trial was conducted. Performance data (time to complete (TTC), rating of perceived exertion (RPE) and mood state), parameters explaining the mechanism of action (endocrine-stress response, oxidative stress and plasma lactate), factors influencing ergogenicity (lifestyle, gender and genetics) and triathlon performance (general health, energy- and nutrient intake, body composition, training regime, side-effects of caffeine withdrawal- and supplementation and hydration status) was collected during two Olympic-distance triathlons (T1 and T2).

Results: Twenty six Caucasian triathletes ($N_m=14$, $N_f=12$) participated (age: 37.8 ± 10.6 years, habitual caffeine intake: 412.7 ± 504.8 mg/day, percentage body fat: 14.5 ± 7.2 %, training/week: 12.8 ± 4.5 hours). There was a 3.7% reduction in swim time (33.5 ± 7.0 vs. 34.8 ± 8.1 minutes) ($p=0.05^*$) and a 1.3% reduction in the overall time to complete the triathlon (149.6 ± 19.8 vs. 151.5 ± 18.6 minutes) ($p=0.02^*$) in the caffeine group. Caffeine did not statistically influence mood state ($p=0.72$) or RPE ($p=0.87$), however, a trend was observed for decreased RPE values in the caffeine group. Caffeine supplementation made no difference to markers of endocrine-stress, except for cortisol, which increased beyond the effect observed from exercise ($p=0.00^*$). Oxidative stress was more pronounced in the caffeine group, as seen with elevated leukocyte ($p=0.05^*$), lymphocyte ($p=0.05^*$) and monocyte ($p = 0.05^*$) counts. Caffeine facilitated greater blood lactate accumulation ($p=0.04^*$). Lifestyle, menstrual cycle, menopause, oral contraceptive use and *CYP1A2* gene polymorphisms did not statistically influence the effect of caffeine supplementation on triathlon performance. The mean energy- and nutrient intake two days before T1 and T2 was low for energy (36.5 ± 17.6 and 38.9 ± 18.2 kcal/kg BW), estimated energy availability ($_{est}EA$) (27.9 ± 28.0 and 28.8 ± 25.6 kcal/kg fat free mass) and carbohydrate (CHO) intake (4.1 ± 1.6 and 4.6 ± 2.5 g/kg body weight (BW)) compared to recommendations. The pre-event meal was low in CHO (0.7 ± 0.4 and 0.7 ± 0.5 g/kg BW) and only 62% ($N=16$) ingested a carbohydrate-electrolyte solution during T1 (CHO: 1.6 ± 2.3 g/kg BW) and T2 (CHO: 0.7 ± 0.4 g/kg BW). Eighty-five percent ($N=22$) used supplements. Seventy-two percent of pre-

menopausal ($N_{f \text{ pre-men}}=5$) and 40% of post-menopausal ($N_{f \text{ post-men}}=2$) females were osteopenic. Of the males, 18% ($N_{m<50} = 2$) had low anterior-posterior spine BMD and 33% ($N_{m>50} = 1$) were osteopenic. Caffeine withdrawal presented as headaches (46%, $N=12$) and flu-like symptoms (38%, $N=10$). Side effects of caffeine experienced included shakiness (42%, $N=11$), heart palpitations (38%, $N=10$) and gastrointestinal disturbances (38%, $N=10$). Plasma volume and hydration was not influenced ($p=0.70$).

Conclusion: Caffeine enhanced triathlon performance, but the effect was not as pronounced as seen in previous laboratory trials and did not affect RPE or mood state. Caffeine supplementation augments the endocrine-stress response by further increasing cortisol levels beyond that resulting from endurance exercise and it induces leukocytosis, neutrophilia and lymphocytosis, suggesting the primary ergogenic effect of caffeine may result due to stimulation of both the central and autonomic nervous systems. Lifestyle, gender and genetics did not significantly influence caffeine's effect on triathlon performance in this cohort. The subjects had low energy, estEA and carbohydrate intake and a high prevalence of osteopenia.

OPSOMMING

Agtergrond: Voldoende bewyse rakende die ergogeniese effek van kaffeïen gedurende uithouvermoë oefening bestaan. Enkel sportsoorte, laboratorium studies en ongeskikte navorsingsontwerpe bevraagteken die toepaslikheid van hierdie studies op driekamp prestasie.

Doelwitte: Die hoofdoelwitte van die studie was om i) die verbetering van prestasie of ergogeniese effek van kaffeïen supplementasie tydens 'n driekamp kompetisie waar te neem; ii) om verskeie parameters wat die ergogeniese effek van kaffeïen supplementasie deels te verduidelik te ondersoek, iii) om moontlike faktore wat die ergogeniese effek van kaffeïen supplementasie kan beïnvloed te ondersoek en iv) om moontlike faktore wat Olimpiese-afstand driekamp prestasie kan beïnvloed te ondersoek.

Metodes: 'n Dubbel-blinde, lukrake, oorkruis, gekontroleerde, kliniese veldproef is uitgevoer. Prestasie data (tyd om die driekampe te voltooi, waargenome inspanning en gemoedstoestand), parameters wat moontlik die aksie van kaffeïen kan verduidelik (endokrien-stress respons, oksidatiewe stress en plasma laktaat), faktore wat die ergogeniese effek van kaffeïen kan beïnvloed (lewensstyl, geslag en genetika) en faktore wat moontlik driekamp prestasie kan beïnvloed (algemene gesondheid, energie- en nutriëntinname twee dae voor en op die dag van die driekampe, liggaamsamestelling en beëindigtheid, oefening twee dae voordie driekampe, nuwe-effekte van kaffeïen onttrekking- en supplementasie en hidrasie status) is ingesamel tydens twee Olimpiese afstand driekampe (T1 en T2).

Resultate: Ses-en-twintig Kaukasiese driekamp atlete ($N_m=14$, $N_f=12$) is ingesluit (ouderdom: 37.8 ± 10.6 , daaglikse kaffeïen inname: 412.7 ± 504.8 mg/dag, % liggaamsvet: $14.5 \pm 7.2\%$, oefening/week: 12.8 ± 4.5 uur). Daar was 'n 3.7% afname in swem tyd (33.5 ± 7.0 teenoor 34.8 ± 8.1) ($p=0.05^*$) en 'n 1.3% afname in totale tyd om die driekampe te voltooi (149.6 ± 19.8 teenoor 151.5 ± 18.6) ($p=0.02^*$) in die kaffeïen groep. Kaffeïen het nie 'n statisties beduidende effek op die gemoedstoestand ($p=0.72$) of die waargenome inspanning ($p=0.87$) gehad nie, maar 'n tendens is waargeneem vir laer waargenome inspanningswaardes in die kaffeïen groep. Kaffeïen het geen verskil gemaak aan parameters van die stres respons nie, behalwe vir kortisol, wat verhoog het bo- en behalwe die effek van oefening ($p=0.00^*$). Oksidatiewe stres was meer uitgesproke in die kaffeïen groep, soos waargeneem deur verhoogde witbloedsel ($p=0.05^*$), limfosiet ($p=0.05^*$) en neutrofiel ($p=0.05^*$) tellings. Kaffeïen fasiliteer die verhoging in bloedlaktaat vlakke ($p=0.04^*$). Lewensstyl, menstruele siklus, menopause, orale voorbehoedmiddel gebruik en *CYP1A2* geen polimorfismes het geen beduidende effek op die vermoë van kaffeïen om driekamp prestasie te beïnvloed gehad nie. Die gemiddelde energie- en nutriëntinname twee dae voor T1 en T2 was laer as die aanbevelings vir energie (36.5 ± 17.6 en 38.9 ± 18.2 kcal/kg LG), geskatte

energie beskikbaarheid (29.9 ± 28.0 en 28.8 ± 25.6 kcal/kg vetvrye massa) en koolhidraat (CHO) inname (4.1 ± 1.6 en 4.6 ± 2.5 g/kg LG). Die voor-driekamp ete was laag in CHO (0.7 ± 0.4 en 0.7 ± 0.5 g / kg LG) en slegs 62% ($N=16$) het 'n koolhidraat-elektroliet oplossing tydens T1 (CHO: 1.6 ± 2.3 g/kg LG) en T2 (CHO: 0.7 ± 0.4 g/kg LG) ingeneem. Vyf-en-tagtig persent ($N=22$) gebruik dieetaanvullings. Twee-en-sewentig persent van die pre-menopausale ($N_{f \text{ pre-men}}=5$) en 40% van die post-menopausale ($N_{f \text{ post-men}}=2$) vroue het osteopenie volgens die totale liggaams been mineraal digtheid. Van die mans, het 18% ($N_{m<50} = 2$) met lae beëindigtheid van die anterior-posterior spina en 33% ($N_{m>50} = 1$) met osteopenie gepresenteer. Waargenome ontrekkingsimptome van kaffeïen was hoofpyn (46%, $N=12$) en griepagtige simptome (38%, $N=10$) en nuwe-effekte was bewegings (42%, $N=11$), hartkloppings (38%, $N=10$) en spysverteringskanaal verstourings (38%, $N=10$). Plasma volume en hidrasie was nie beïnvloed nie ($p=0.70$).

Gevolgtrekking: Kaffeïen verbeter driekamp prestasie, maar die effek is nie so uitgesproke soos waargeneem tydens laboratorium studies nie en het nie 'n beduidende effek op waargenome inspanning of gemoedstoestand getoon nie. Kaffeïen verhoog die stres respons deur die verdere verhoging van kortisol vlakke, bo- en behalwe vlakke waargeneem tydens uithouvermoë oefening en verhoog witbloedsel, limfosiet en neutrofiel tellings. Dit dui daarop dat die primêre ergogeniese effek van kaffeïen suplementasie moontlik as gevolg van stimulasie van beide die sentrale en autonome sensuïesistels voorkom. Lewensstyl, geslag en genetica het nie 'n beduidende effek op die ergogeniese vermoë van kaffeïen getoon in hierdie studiepopulasie nie. Die deelnemers het 'n lae energie, geskatte energie beskikbaarheid en koolhidraatinname gehad. Die studiegroep het 'n hoë prevalensie van osteopenie.

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This dissertation is dedicated to the loving memory of my father:

Stefanus Albertus Bam • 25/02/1950 – 15/11/1996

CONTRIBUTIONS BY PRINCIPAL AND FELLOW RESEARCHERS

The principal researcher (S Potgieter) developed the idea and the protocol. The principal researcher planned the study, sourced funding, undertook data collection (with the assistance of field workers), captured the data for analyses, analysed the data with the assistance of a statistician (Prof DG Nel), interpreted the data and drafted the dissertation. Prof C Smith, Dr H Wright and Prof L Warnich (supervisors) provided input at all stages and revised the protocol and dissertation. Dr L Cassim, founder and managing member of Layla Cassim ERS (Education, Research, and Science) Consultants CC language edited the dissertation.

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LIST OF ABBREVIATIONS

A	Adenine
ACSM	American College of Sports Medicine
ADA	American Dietetic Association
<i>AHR</i>	Aryl hydrocarbon receptor gene
AI	Adequate Intake
AT	Anaerobic Threshold
% BF	Percentage Body Fat
BMD	Bone Mineral Density
BMI	Body Mass Index
Bp	Base pair
C	Cytosine
CHO	Carbohydrate
CNS	Central Nervous System
CYP450	Cytochrome P450
DNA	Deoxyribonucleic Acid
dNTPs	Deoxynucleotide triphosphates
DRI	Dietary Reference Intake
DXA	Dual energy X-ray Absorptiometry
_{est} EA	Estimated Energy Availability
EAR	Estimated Average Requirement
EEE	Energy Expended in Exercise
EI	Energy Intake
FBC	Full Blood Count
FFA	Free Fatty Acids
FL	Finish Line
G	Guanine
gDNA	Genomic Deoxyribonucleic Acid
g/kg BW	grams per kilogram Body Weight
g/l	grams per litre
HR	Heart Rate
IOC	International Olympic Committee
ISSN	International Society of Sport Nutrition
ITU	International Triathlon Union
Kb	Kilobase
km	kilometres

kJ	kilojoules
kJ/kg BW	kilojoules per kilogram Body Weight
MET	Metabolic Equivalent of Task
mg/kg BW	milligrams per kilogram Body Weight
mmol/l	millimole per litre
N	Total sample
N_c	Total caffeine
N_{cf}	Total caffeine female
N_{cm}	Total caffeine male
N_f	Total female sample
N_m	Total male sample
nmol/l	nanomole per litre
N_p	Total placebo
N_{pf}	Total placebo female
N_{pm}	Total placebo male
NSRI	National Sea Rescue Institute
OD	Olympic-distance
OTC	Over the Counter
PB	Personal Best
POMS	Profile Of Mood State
RDA	Recommended Dietary Allowance
RER	Respiratory Exchange Ratio
RPE	Rating of Perceived Exertion
SNP	Single nucleotide polymorphism
T	Thymine
$t_{1/2}$	Half-life of a drug
T_A	Annealing temperature
T1	Triathlon 1
T2	Triathlon 2
% TE	Percentage of Total Energy
TP	Time Point
TSA	Triathlon South Africa
TT	Time Trial
TTE	Time to Exhaustion
UL	Upper Limit
umol/l	micromole per litre
ug/l	microgram per litre

V	Volt
WADA	World Anti-Doping Association
WHO	World Health Organization
WPTA	Western Province Triathlon Association

GLOSSARY

Allele	each of two or more alternative forms of a gene that arise by mutation and are found at the same place on a chromosome (1)
Amenorrhea	abnormal absence of menstruation (cycle length >90 days) (1)
Drafting (slipstreaming)	technique where two vehicles or other moving objects (bicycles) are caused to align in a close group reducing the overall effect of drag due to exploiting the lead object's slipstream. In triathlon, drafting refers to completing the physical activity behind another athlete completing the same activity in a "sheltered" position (2)
Drug	any chemical substance that has an effect on the body (3)
Estimated energy availability	dietary energy intake minus energy expended in exercise ($EA = EI - EEE$) and is expressed in kcal/kg fat free mass (FFM) (4)
Ergogenic	intended to enhance physical performance, stamina, or recovery (1)
Eumenorrhea	normal menstruation, a normal menstrual cycle is typically between 21 and 35 days between menstrual periods (1)
Follicular phase	also known as the proliferative phase, it is the phase of the menstrual cycle during which follicles in the ovary mature and end with ovulation (1)
Genotype	the genetic constitution of an individual organism (1)
Glycogen	multi-branched polysaccharide that serves as a form of energy storage (storage form of glycogen) (5)
Gynaecological age	current age minus the age of menarche (number of years since menarche) (6)
Habituation	the gradual acclimatisation or familiarisation with a substance or phenomenon to the point at which it goes unnoticed (7)
Half-life of a drug	time taken for concentration of a drug in the blood to fall by half its original value (3)

Heterozygotes	an individual having two different alleles of a particular gene or genes (1)
Homozygous	an individual having two identical alleles of a particular gene or genes (1)
Luteal phase	also known as the secretory phase, it is the latter phase of the menstrual cycle and begins with the formation of the corpus luteum and ends in either pregnancy or luteolysis (structural and functional degradation of the corpus luteum) (1)
Menarche	the first occurrence of menstruation (1)
Menopause	permanent cessation of the primary functions of the human ovaries; the ripening and release of ova and the release of hormones that cause both the creation of the uterine lining and the subsequent shedding of the uterine lining. Typically occurs in women in midlife, during their late 40s or early 50s, and signals the end of the fertile phase of a woman's life (1)
Metabolic equivalent of task	oxygen cost of energy expenditure measured at supine rest (1 MET = 3.5 ml O ₂ per kg body weight per minute); multiples of METs are used to estimate the oxygen cost of activity (7)
Oligomenorrhea	abnormal menstrual cycle, cycle length between 35-90 days or < 10 cycles/12 months (1)
Olympic-distance triathlon	an athletic contest consisting of three different events, typically swimming, cycling, and long-distance running, an Olympic or standard distance triathlon comprises of a sequential swim (1.5 km), swim-to-cycle transition, cycle (40 km), cycle-to-run transition and run (10 km) (2, 8)
Osteopenia	a medical condition in which the protein and mineral content of bone tissue is reduced, but less severely than in osteoporosis (1)
Osteoporosis	a medical condition in which the bones become brittle and fragile from loss of tissue, typically as a result of hormonal changes, or deficiency of calcium or vitamin D (1)

Pharmacodynamics	the branch of pharmacology concerned with the effects of drugs and the mechanism of their action (1, 3)
Pharmacokinetics	the branch of pharmacology concerned with the movement of drugs within the body (absorption, distribution, metabolism and elimination) (1, 3)
Phenotype	the set of observable characteristics of an individual resulting from the interaction of its genotype with the environment (1)
Phlebotomy	obtaining blood from a vein (7)
Rating of perceived exertion	exertion is the expenditure of energy by skeletal muscles, the intensity of this action can be measured by the rate of which oxygen is expended, heat is produced and heart rate, a frequently used term is rating of perceived exertion or RPE-scale, also known as the Borg scale, which is use of a scale to indicate a quantitative feeling of fatigue (9)
Single nucleotide polymorphism	variation in a single base pair in a DNA sequence (1)
Stress response	the physiological response of the whole animal, in contrast to cellular stress responses, the induction and regulation of which are distinct from responses of the intact animal. Stimuli that induce stress responses are stressors, and common stressors include psychological, physical, and drug or chemical stimuli. Activation of this physiological response almost always is associated with activation of the hypothalamic-pituitary-adrenal (HPA) axis and the sympathetic nervous system, leading to changes in the concentrations of stress-related mediators (10)
Sub-maximal exercise	an exercise test halted at a predetermined point that is less than the maximal exercise capability of the subject, usually at a particular percentage of the maximal heart rate or after a set time interval (7)
Time trial	a test of a competitor's individual speed over a set distance, especially a cycling race in which competitors are separately timed, an exercise designed to test the time needed for a task or activity (1)

Transition	the process or a period of changing from one state or condition to another (1), in the context of triathlon, the changeover from the swim to the cycle and from the cycle to the run sections of the triathlon (2, 8)
Triathlon 1	first time trial of the current research study, comprising of an Olympic-distance triathlon (swim (1.5 km), swim-to-cycle transition, cycle (40 km), cycle-to-run transition and run (10 km) held at Gordon's Bay beach, Western Cape, South Africa on 22 May 2011
Triathlon 2	second time trial of the current research study, comprising of an Olympic-distance triathlon (swim (1.5 km), swim-to-cycle transition, cycle (40 km), cycle-to-run transition and run (10 km) held at Gordon's Bay beach, Western Cape, South Africa on 5 June 2011
T-score	relevant measure when screening for osteoporosis, the bone mineral density (BMD) at the site when compared to the young normal reference mean, it is a comparison of a patient's BMD to that of a healthy thirty-year-old of the same sex and ethnicity, value is used in post-menopausal women and men over aged 50 because it better predicts risk of future fracture (11) the criteria of the World Health Organization are; normal is a T-score of -1.0 or higher, osteopenia is defined as between -1.0 and -2.5 and osteoporosis is defined as -2.5 or lower, meaning a bone density that is two and a half standard deviations below the mean of a thirty year old man/woman (12)
Vacutainer	blood collection tube that is a sterile glass or plastic tube with a closure that is evacuated to create a vacuum inside the tube facilitating the draw of a predetermined volume of liquid (7)
Venipuncture	puncture of a vein through the skin in order to withdraw blood for analysis (7)
VO ₂ max	the maximum or optimum rate at which the heart, lungs, and muscles can effectively use oxygen during exercise, used as a way of measuring a person's individual aerobic capacity (1)

Z-score

the comparison to the age-matched normal and is usually used in cases of severe osteoporosis, this is the number of standard deviations a patient's BMD differs from the average BMD of their age, sex, and ethnicity, this value is used in premenopausal women, men under the age of 50, and in children, it is most useful when the score is less than 2 standard deviations below this normal (11)

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CHAPTER 1: INTRODUCTION

1.1 Background, research contextualisation and problem statement

There is abundant evidence regarding the ergogenic effect of caffeine during endurance exercise and general consensus amongst published reports about the ergogenic properties of caffeine. Most of these studies have investigated the effect of caffeine on performance in cycling, running, swimming, rowing and skiing, but none of these studies has considered the effect of caffeine supplementation on a combination of these events, specifically triathlon performance.

The present research study focused on four distinct aspects in terms of caffeine supplementation and triathlon performance. This study investigated the possible ergogenic effect of caffeine supplementation on real life Olympic-distance triathlon performance, including an evaluation of several parameters that could in part explain why caffeine supplementation is ergogenic. In addition, it provides an in depth investigation of possible factors influencing the ergogenicity of caffeine supplementation on Olympic-distance triathlon as well as possible confounding factors influencing triathlon performance. The rationale for investigating these aspects will be discussed briefly in this Chapter.

1.2 The possible ergogenic effect of caffeine supplementation on real life Olympic-distance triathlon performance

Published studies have investigated the ergogenic properties of caffeine supplementation during or before various time trial performance tests in swimming, cycling, running and rowing (13-18); cycling and running time to exhaustion protocols (19-30); ratings of perceived exertion (15, 27, 29, 31, 32); prolonged cycling at 60-70% VO_2 max (27, 29, 30) and high intensity intermittent team sport (33, 34). It was concluded from these studies that caffeine supplementation decreases the time to complete time trial events, increases endurance when exercising to exhaustion (i.e. increases the time to fatigue), decreases ratings of perceived exertion and increases prolonged sub-maximal cycling performance.

Studies that found no beneficial effect of caffeine supplementation on sports performance involved non-endurance exercise tests or events. These studies looked at athletic agility (35), weight training (36), exercise in extreme conditions (37), repeated sprints in team-sport athletes (38), maximal ability to generate power, reduction in fatigue during high-intensity dynamic exercise (39) or repeated bouts of short term intense exercise measured by Wingate tests (40). Other studies showing similar results were not standardised in terms of nutrition or environmental factors. Therefore, although these studies all addressed specific problems and provide valuable information about performance under particular conditions,

these reported results are not directly applicable to a competitive endurance event like a triathlon.

There are also several limitations in the research design and methodology employed in many of these published studies on the effects of caffeine supplementation on performance. In most of these studies, for example, performance was measured by means of “time to exhaustion” protocols. Although this provides valuable laboratory information on the effect of caffeine supplementation, it is not an accurate reflection of exercising in the field. There is a greater coefficient of variation when a subject performs a time to exhaustion protocol in comparison to a time trial performance (41-43). Time to exhaustion does not measure the true exercise performance benefit of caffeine as no sport requires an athlete to endure more or complete a longer distance than his/her competitors (41, 42).

Another limitation is that several studies (28-30) also use protocols in which athletes exercise for longer periods of time (a total of 120-240 minutes) at constant sub-maximal intensity, which is not applicable to a race situation. The few field studies published on the ergogenic effect on caffeine are not applicable to long-duration endurance exercise, (such as a triathlon), under normal environmental conditions (13, 41, 44-46).

A triathlon specifically requires an athlete to complete the set amount of work or distance in the shortest amount of time; it is thus a time trial performance. Triathletes experience a variety of environmental factors, functional demands and other factors such as drafting position, power output and the selection of cycling cadence, to a greater extent than encountered in single sporting events of equal duration (47, 48).

There is a difference between the elements affecting exercise performance during a race and those acknowledged during laboratory experiments. One of the biggest variances is related to the stability of power output in experimental studies when compared to pacing strategies used during competition, particularly when the competitive stakes are high (49). Field studies allow for more strength of performance measures because field studies include real airflow and ground resistance, real life pacing tactics, fluctuations in the environment and course over the event and the effect of rivalry and other extrinsic factors (42). Laboratory-based studies with very close control over the environment and the athlete have shown that caffeine has an effect on metabolism and exercise performance (42). This cannot, however, always be extrapolated to real-life events.

Most published studies on the ergogenic effect of caffeine supplementation use healthy or well-trained individuals in more widely practised disciplines such as running or cycling, and usually do not focus specifically on triathletes. Therefore, the present study aimed to investigate the possible ergogenic effect of caffeine supplementation on Olympic-distance triathlon in near real life conditions, specifically focussing on the effect of caffeine supplementation on triathlon performance, rating of perceived exertion and changes in mood state.

1.3 Parameters that could in part explain why caffeine supplementation is ergogenic

Available research on the mechanism(s) by which caffeine supplementation results in an ergogenic effect is controversial and more research in this field is needed to fully understand and comprehend the mechanism(s) by which caffeine enhances performance as well as the extent of improvement in endurance performance. This is of particular importance when research is conducted in the field compared to the laboratory setting, as the biochemical effect of caffeine supplementation may be altered when an athlete is removed from the controlled laboratory environment and is highly motivated and aroused to compete in a real-life competition (50, 51). Due to the fact that exercise and caffeine supplementation can both be seen as independent stressors, the real-life setting as simulated in the present study provides valuable information regarding the endocrine-stress response (cortisol, prolactin, dehydroepiandrosterone sulphate and testosterone), oxidative stress response (total and differential white blood cell count) and the adrenergic response (lactate) that have all been associated with the ergogenic effect of caffeine supplementation. These parameters result either from the direct effect of caffeine on the central nervous system or indirectly from the effect of caffeine on the hypothalamic-pituitary-adrenal axis (HPA-axis), autonomic nervous system and immune response (52, 53). Further biophysiological rationals for including these parameters is presented in Chapter 2.

1.4 Possible factors influencing the ergogenicity of caffeine supplementation on Olympic-distance triathlon performance

Various factors such as lifestyle, gender and genetics can affect the pharmacokinetics of caffeine and thus influence the ergogenic effect of caffeine.

First, lifestyle factors important to consider include caffeine habituation, where increased doses of caffeine are needed to elicit the same effect of caffeine supplementation. The pre-

event dietary intake, smoking and excessive alcohol intake may also affect the ergogenicity of caffeine supplementation (54-63).

Second, the influence of gender should be accounted for. There are a limited number of studies differentiating between subjects and/or results on a gender basis. A large amount of the existing research on caffeine consumption in sport or exercise has only included male athletes. In a 2009 systematic review, only 10 of the 29 trials included women in the study population, of which two studies tested only female subjects and statistical analysis in the other eight trials did not distinguish between males and females (41). This is of importance, as gender, and more specifically the phase of the menstrual cycle, menopause and oral contraceptive use has the potential to influence caffeine metabolism and thereby its effect on exercise performance. Caffeine excretion is slower during the late luteal phase of the menstrual cycle and the use of oral contraceptive medication increases plasma caffeine concentrations. Menopause has also been shown to influence caffeine metabolism.

Third, the risk and/or benefit of caffeine supplementation depend largely on the metabolism thereof, which is genetically determined. The enzyme that is responsible for the breakdown of caffeine in the liver (*CYP1A2*) is genetically determined and therefore some people may be more sensitive to the use of caffeine than others. This may account for the contradictions found in the current literature with regards to the increased or decreased risk of adverse health effects and the effect thereof on sport performance. The prevalence and influence of *CYP1A2* gene polymorphism can therefore also potentially affect the ergogenicity of caffeine supplementation.

It is evident that various factors, such as lifestyle, gender and genetics can affect the pharmacokinetics of caffeine. It is thus imperative when studying the performance enhancing effect of caffeine supplementation to take these factors into consideration.

Additionally, in order to attribute the ergogenic effect seen during endurance exercise to caffeine supplementation, one has to also consider other possible confounding factors that can influence Olympic-distance triathlon performance, apart from the abovementioned factors influencing the ergogenicity of caffeine supplementation.

1.5 Possible confounding factors influencing Olympic-distance triathlon performance

Well-controlled field research studies should ideally control for not only the factors influencing the ergogenicity of caffeine supplementation, but also for factors that can influence endurance performance above and beyond the intervention. General health, dietary intake, body composition and training in the days leading up to the event, symptoms of caffeine withdrawal and side-effects of caffeine supplementation, as well as hydration are important aspects to consider in this regard.

There are at least two approaches toward conducting research in the field setting. One can either conduct the research at real life conditions, whilst measuring and accounting for all possible confounding factors or one can control for these confounding factors. After long deliberation, the researcher decided to make the brave decision to conduct a clinical trial in real life circumstances, whilst noting all factors that could possibly influence performance. It is the opinion of the author that this type of approach leads to wider applicability and increased practical application of the results. It is important to test the effect of ergogenic aids in the situation in which it will ultimately be used. Although this approach would dilute the actual effect of the ergogenic aid, this is the actual effect that will be observed when used in real life situations, such as in the present study. In the South African context this is of particular importance as athletes have either limited resources or knowledge regarding optimal pre-race strategies. Although this situation is not ideal, it is reality in South Africa.

Therefore, this research protocol allowed for subjects to follow their own preparational, including nutritional strategies and the use of their own equipment in order to imitate real-life practices. Because of the major impact of dietary intake and body composition on exercise and more specifically endurance performance, this study also focussed on the comprehensive assessment of the dietary intake and body composition and its effect on triathlon performance.

1.6 Aim and objectives

1.6.1 Aim

Given the limitations explored above and the fact that most research on caffeine supplementation has been conducted in a laboratory setting, the need arose to conduct a double-blind, randomized, crossover, controlled, clinical field trial, utilizing optimal performance assessment tools, to establish the true effect of caffeine supplementation on provincial-level male and female Olympic-distance triathletes and triathlon performance in

the Western Cape, South Africa. The use of a double-blind research design and appropriate statistical methods is imperative, because the margin between the athletes winning and losing can be measured in seconds.

Therefore, the main aims of this study were to i) investigate the performance-enhancing or ergogenic effect of caffeine supplementation during a real-life triathlon competition; ii) evaluate several parameters that could in part explain why caffeine supplementation is ergogenic, iii) investigate possible factors influencing the ergogenicity of caffeine supplementation and iv) investigate possible confounding factors influencing Olympic-distance triathlon performance.

1.6.2 Research objectives

Data was collected during two triathlons held 14 days apart (22 May and 5 June 2011), at the same venue in Gordon's Bay, Western Cape Province, South Africa. Both triathlons consisted of a sequential 1.5 km swim, 40 km cycle and 10 km run (T1 and T2).

To investigate the performance-enhancing or ergogenic effect of caffeine supplementation during a real-life triathlon competition with regard to:

- i) Effect on triathlon performance by measuring the time to complete the swim, cycle, run and overall time to complete the Olympic-distance triathlon;
- ii) Rating of perceived exertion during and after an Olympic-distance triathlon; and
- iii) Mood state before and after an Olympic-distance triathlon

Evaluation of parameters that could in part explain why caffeine supplementation is ergogenic, with specific focus on the following:

- i) Endocrine-stress response (serum cortisol, prolactin, testosterone and dehydroepiandrosterone-sulphate (DHEAs));
- ii) Infection, inflammation and oxidative stress (total and differential white cell count);
- iii) Plasma lactate

To investigate possible factors influencing the ergogenicity of caffeine supplementation, such as:

- i) Lifestyle habits including caffeine habituation and the pre-event meal;
- ii) The influence of gender and more specifically the effect of the phase of the menstrual cycle, oral contraceptive use and menopause on the ergogenic effect of caffeine supplementation;

- iii) The prevalence and influence of *CYP1A2* gene polymorphism on the ergogenic effect of caffeine supplementation;

To investigate possible confounding factors influencing exercise or specifically Olympic-distance triathlon performance, such as:

- i) General health
- ii) Energy- and nutrient intake two days before as well as dietary strategies followed on the race day
- iii) Body composition and bone mineral density
- iv) Training two days before race day;
- v) Side effects of caffeine withdrawal
- vi) Side effects of caffeine supplementation
- vii) Hydration status and changes in plasma volume (plasma albumin, haematocrit and hemoglobin)

1.6.3 Hypothesis

Four hypotheses were subsequently investigated, in a sample of provincial-level, Olympic-distance triathlon athletes in the Western Cape, South Africa. These hypotheses are outlined below.

- i) Double-blind, randomized, crossover, controlled, clinical field trials and not only laboratory experiments are necessary to establish the real effect of caffeine supplementation on the performance time, rating of perceived exertion and mood state before, during and after an Olympic-distance triathlon.
- ii) The endocrine-stress response, oxidative stress and plasma lactate levels could potentially explain the ergogenic effect of caffeine supplementation;
- iii) Lifestyle, gender and genetics influence the ergogenicity of caffeine supplementation.
- iv) General health, energy- and nutrient intake, body composition, training status, caffeine withdrawal symptoms and side effects of caffeine supplementation influence Olympic-distance triathlon performance.

The conceptual framework shown in Figure 1.1 below was established according to these research arguments.

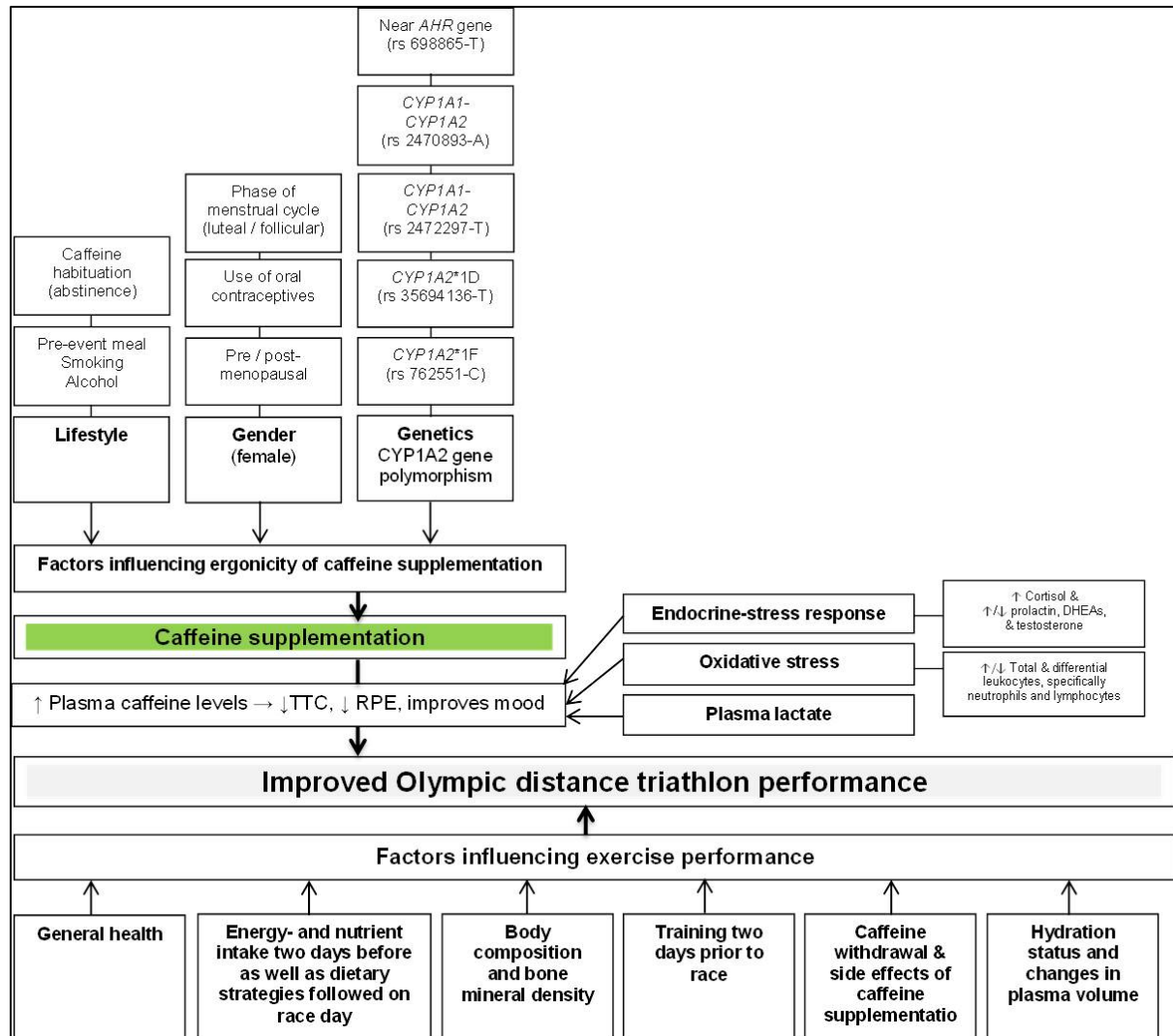


Figure 1.1 Conceptual framework illustrating the research arguments in this study

TTC: time to complete; RPE: rating of perceived exertion; DHEAs: dehydroepiandrosterone sulphate.

1.7 Brief chapter overview

This dissertation is structured according to the four main aims of the study. First, the possible ergogenic effect of caffeine supplementation with a biophysiological rationale for evaluating several parameters that could potentially explain why caffeine exerts an ergogenic effect, factors influencing the ergogenicity of caffeine supplementation as well as factors that can influence exercise performance. In line with this, Chapter 2 focusses on a comprehensive overview of the literature, followed by a detailed description of the research design and methodology employed is provided in Chapter 3. Chapter 4 presents the results of the study and the statistical analysis of these, while Chapter 5 discusses and interprets the results in the context of current literature on the topic. The dissertation concludes with Chapter 6, which consists of the summary of the main findings and practical application

thereof, the limitations of the study, recommendations for future research, and the concluding remarks.

CHAPTER 2: LITERATURE OVERVIEW

2.1 Introduction

Caffeine (1,3,7-trimethylxanthine, molecular weight 194.2) (54, 64), taken in moderate dosages of 200-300 mg/day is not harmful to general health (65, 66). It is widely found in non-prescription medication (30-200 mg/tablet), coffee (40 – 180 mg/150ml), Ceylon tea (24 – 50 mg/150ml), soft drinks (15 – 40 mg/180ml), chocolate bars (1 – 36 mg/ 28g), cocoa (2 – 10 mg/150ml) (57, 67, 68), energy gels and energy drinks (30-85mg/250ml) (42, 54, 57, 64).

Although caffeine is not a nutrient, such as carbohydrate (CHO), protein, fat, vitamins or minerals, it is a commonly-used dietary constituent. Of all the non-alcoholic drinks, coffee is second to water with regards to consumption worldwide (66). Every year, the global population consumes approximately 500 billion cups of coffee (66). Coffee is also the second biggest trade commodity across the globe after petroleum (66). Habitual caffeine consumption from coffee, tea and cocoa has been reported in almost 80% of the total population, with South Africa (40 mg/person/day) at the lower end of consumption in comparison to countries such as the Netherlands (414 mg/person/day), Sweden (407 mg/person/day), Norway (400 mg/person/day), Denmark (390 mg/person/day), Finland (329 mg/person/day) and Germany (313 mg/person/day) (57). Caffeine consumption from all sources can be estimated to around 70-76 mg/person/day worldwide (57).

Caffeine is a substance with no nutritional value and has attracted the consideration of many elite and amateur athletes as an ergogenic aid (69). Self-reported mean daily caffeine intake amongst athletes varies widely, with the mean daily intake of six Canadian male recreational athletes (running or cycling five times a week for more than three months) found to be as high as 761.3 ± 11.8 mg (26). However, a study by Chester *et al.* (2008) of British athletes stated that although most of the cyclists used caffeine, levels remained below 200 mg/day (70). In South Africa, 42% of triathletes living in the Western Province consume herbal supplements with or without caffeine on a daily basis and only 4% consume caffeine on a habitual basis as reported by the researcher in a previous study on this group of athletes (71).

A recent review of the risks and benefits of coffee/caffeine consumption concluded that caffeine can have a significant effect on the body, despite controversy and conflicting research (66). Moderate amounts of caffeine can increase energy substrate availability and daily energy expenditure; reduce fatigue and the sense of effort associated with physical activity; increase physical, motor and cognitive performance; increase alertness, wakefulness and feelings of energy; decrease mental fatigue; quicken and increase the accuracy of reactions; enhance concentration and short-term memory; improve the ability to

focus; solve problems requiring reasoning and make correct decisions; and increase cognitive function capabilities and neuromuscular coordination (65).

2.2 Ergogenic effect of caffeine supplementation

2.2.1 Caffeine and sport legislation

Caffeine used to be on the International Olympic Committee's (IOC's) list of prohibited substances, but with the provision that the urinary caffeine levels exceeded 12 mg/l. However, on 1 January 2004 the World Anti-Doping Association (WADA) removed the substance from the prohibited list and instead placed it in the organisation's monitoring program (64, 72-74). It is impractical for caffeine to be listed as a banned substance, as the dose needed to elicit a performance-enhancing effect is relatively small (3-6 mg/kg body weight) and an athlete can easily ingest this amount in common foodstuffs (64, 72-74).

The American National Collegiate Athletic Association (NCAA) currently uses a urinary caffeine cut-off value of 15 mg/l (72, 73) and the South African Institute for Drug Free Sports uses the WADA guidelines in South African anti-doping rules and policies. Furthermore, only 5% of an administered dose of caffeine is eliminated in urine. Acute dosages of up to 9-13 mg/kg body weight can thus be administered without reaching a urinary caffeine level of 12 mg/l (64, 72, 73).

2.2.2 Evidence in support of the ergogenic effect of caffeine

Performance in several types of exercise, including endurance exercise, high-intensity team sport, strength-power performance and special force operations, has been shown to be significantly increased by the ingestion of caffeine (72).

Table 2.1 summarizes various studies on the ergogenic properties of caffeine supplementation during or before various endurance exercises, such as time trial swimming, cycling, running and rowing, cycling and running time to exhaustion protocols, ratings of perceived exertion, prolonged cycling at 60-70% VO_2 max, and high intensity intermittent team sport.

Table 2.1 Studies examining the effect of caffeine supplementation on endurance sport performance (cycling, running, swimming or rowing)

Reference	Participants	Supplementation / Intervention	Outcome measured	Conclusion
Desbrow <i>et al.</i> (2012)	16 male cyclists Habitual caffeine consumers Abstained 24 hours prior	3 & 6 mg/kg 90 minutes prior	Set amount of work @ 75% peak sustainable power output for 60 minutes	Similar ↑ performance in both groups No effect on RPE
Roelands <i>et al.</i> (2011)	8 male cyclists	6 mg/kg 60 minutes prior	Cycled for 60 minutes @ 55% W_{max} , followed by TT (30°C)	No change in performance
Ganio <i>et al.</i> (2011)	11 male cyclists Low daily users Abstained 3 days prior	3 mg/kg 60 minutes prior & again 45 minutes into exercise	90 minutes @ 65% VO_2 max, followed by 15 minute TT	RPE not affected by caffeine ↑ exercise capacity ↓ leg muscle pain in heat
Ganio <i>et al.</i> (2011)	11 male cyclists Habitual and non-users Abstained 3 days prior	3 mg/kg 60 minutes prior & after 45 minutes of exercise	90 minutes @ 65% VO_2 max + 15 minute TT (12°C and 33°C)	↑ performance independent of temperature
Ely <i>et al.</i> (2011)	10 males Non-users	9 mg/kg	Cycle ergometer exercise for 30 minutes @ 50% VO_2 max (40°C, 25% humidity)	No significant change in heat balance
Carr <i>et al.</i> (2011)	6 male & 2 female rowers Abstained 48 hours prior	6 mg/kg 30 minutes prior	2000 m rowing ergometer tests	↑ power
Backhouse <i>et al.</i> (2011)	12 male cyclists Low to moderate consumers Abstained for 60 hours prior	6 mg/kg 60 minutes prior	90 minutes @ 70% VO_2 max	↓ RPE
Simmonds <i>et al.</i> (2010)	6 male cyclists Habitual caffeine users Abstained 24 hours prior	5 mg/kg	Supra-maximal cycle bouts (120% VO_2 max) to exhaustion	Beneficial to supra-maximal cycling performance
Ping <i>et al.</i> (2010)	9 heat-adapted male runners Non-users of caffeine Abstained at least 10 hours	5 mg/kg 60 minutes prior	Running @ 70% VO_2 max (31°C, 70% humidity)	↑ running performance No change in RPE
Hadjicharalambous <i>et al.</i> (2010)	10 male cyclists Abstained 48 hours prior	High fat meal vs. high fat meal + 7.5 mg/kg caffeine	Cycle TTE (10°C & 70% humidity) @ ± 73% VO_2 max	Artificial elevation in plasma FFA no additional ↑ in endurance performance from caffeine
Walter <i>et al.</i> (2009)	20 males	200 mg 30 minutes prior	TTE @ 80% VO_2 max	No difference in TTE
Ivy <i>et al.</i> (2009)	6 male & 6 female cyclists Abstained overnight	160 mg 40 minutes prior	Time to complete standard amount of work equal to 60 minutes cycling @ 70% W_{max}	↑ performance No change in RPE
Desbrow <i>et al.</i> (2009)	9 male cyclists Habitual and non-users Abstained 24 hours prior	1.5 or 3 mg/kg 60 minutes prior	120 minutes steady state cycling @ 70% VO_2 max + TT	No significant ↑ TT performance

Reference	Participants	Supplementation / Intervention	Outcome measured	Conclusion
Candow <i>et al.</i> (2009)	9 male, 8 female Abstained 48 hours prior	2 mg/kg	Run TTE @ 80% VO ₂ max	No difference in TTE, RPE or blood lactate
McNaughton <i>et al.</i> (2008)	6 male cyclists Habitual caffeine consumers Abstained 24 hours	6 mg/kg 120 minutes prior	60 minutes cycling TT	↑ distance cycled during TT ↑ performance
Hogervorst <i>et al.</i> (2008)	24 cyclists Moderate habitual caffeine Abstained overnight	Performance bar with 100 mg caffeine	2.5 hour cycling @ 60% VO ₂ max & TTE @ 75% VO ₂ max	↑ performance & complex cognitive ability during & after exercise
Del Coso <i>et al.</i> (2008)	7 endurance trained males Light caffeine users	6 mg/kg BW 45 minutes prior	Prolonged exercise in heat, pedaled for 120 minutes @ 63% VO ₂ max in hot-dry environment	↑ maximal cycling power When combined with water & CES: ↑ increases maximal leg force ↓ reducing central fatigue
Beck <i>et al.</i> (2008)	31 male	201 mg 45 minutes prior	Running TTE @ 85% VO ₂ max	No effect on running TTE
Demura <i>et al.</i> (2007)	10 males Abstained overnight	6 mg/kg BW 60 minutes prior	Submaximal endurance cycling 60 minutes @ 60% VO ₂ max	↓ RPE No difference in oxygen uptake RER, HR / plasma lactate
Cureton <i>et al.</i> (2007)	16 cyclists Habitual caffeine consumers Abstained the day prior	5.3 mg/kg	Cycled for 135 minutes (60%-75% VO ₂ max) & 15 minute performance ride	Work completed during performance ride ↑, ↓ RPE ↓ maximal voluntary contraction
Hadjicharalambous <i>et al.</i> (2006)	8 male endurance athletes Abstained 48 hours prior	High fat meal vs. high fat meal + 7.0 mg/kg 60 minutes prior	3 constant-load cycling tests @ ± 73% VO ₂ max for 30 minutes <i>et al.</i> (20°C), followed by incremental exercise to fatigue	↓ RPE
	10 male cyclists Abstained 48 hours prior	High fat meal vs. high fat meal + 7.5 mg/kg 60 minutes prior	3 constant-load cycling tests to limit of tolerance at 10°C	↓ RPE did not ↑ performance
Bridge <i>et al.</i> (2006)	8 male distance runners Habitual caffeine consumers Abstained 48 hours prior	3 mg/kg	8 km running TT	↓ 8 km running time
Beedie <i>et al.</i> (2006)	6 male cyclists No abstinence	4.5 mg/kg & 9 mg/kg (placebo masked as caffeine)	Evaluated placebo effect	Placebo effect present
O'Connor <i>et al.</i> (2004)	12 males Low habitual caffeine consumers Abstained 1 week prior	5 / 10 mg/kg 60 minutes prior	30 minutes cycling @ 60% VO ₂ max	↓ leg muscle pain
McLellan <i>et al.</i> (2004)	9 male + 4 female untrained subjects Habitual users	3, 5, 7 mg/kg 90 minutes prior	Cycling at 80% VO ₂ TTE	↑ TTE , regardless of dose

Reference	Participants	Supplementation / Intervention	Outcome measured	Conclusion
Haller <i>et al.</i> (2004)	7 men & 3 females Non-users of caffeine Abstained 24 hours prior	303.8 mg caffeine	RPE	↓ RPE
Doherty <i>et al.</i> (2004)	11 male cyclists Abstained 24 hours prior	5 mg/kg	High intensity cycling performance	↓ RPE ↑ mean power output
Birnbaum <i>et al.</i> (2004)	5 male & 5 female cross country runners Habitual and non-users Abstained 4 days prior	7 mg/kg 60 minutes prior	30 minute run @ 70% VO ₂ max	↑ respiratory efficiency ↓ RPE
Conway <i>et al.</i> (2003)	9 male cyclists & triathletes Abstained 48 hours prior	6 mg/kg 60 minutes prior OR 3 mg/kg, 60 minutes prior & again 3 mg/kg 45 minutes into exercise	Cycled 90 minutes @ 68% VO ₂ max Self-paced TT @ 80% VO ₂ max over 30 minutes	No change in TT performance “tended” to be faster
Bell <i>et al.</i> (2003)	9 recreational male cyclists Habitual caffeine consumers Abstained 12 hours prior	5 mg/kg AM + 2.5 mg/kg PM (5 hours later) OR 5 mg/kg AM OR 5 mg/kg PM 60 minutes prior (AM & PM)	TTE @ 80% VO ₂ max	Re-dosing not necessary to maintain ergogenic effect during subsequent exercise 6 hours later ↓ RPE
Cox <i>et al.</i> (2002)	12 male cyclists/triathletes Habitual caffeine users	6 mg/kg 60 minutes prior and 6 X 1 mg/kg every 20 minutes	120 minutes steady state cycling @ 70% VO ₂ max + 7 kJ/kg TT	↑ TT performance independent of timing of intake
	8 highly trained male cyclists or triathletes Occasional – habitual intake (150mg /day)	3 X 5 ml decaffeinated cola drink (6% CHO) OR caffeinated cola drink (13mg/100ml) (6% CHO) OR decaffeinated cola drink (11% CHO) OR caffeinated (13mg/100ml) cola drink (11% CHO)		Replacing sports drink with coca-cola during latter stages ↑ endurance performance ↓ RPE
Bell <i>et al.</i> (2002)	10 male & 2 female runners Habitual and non-users 6 Abstained 24 hours prior	4 mg/kg 90 minutes prior	10 km running on treadmill (wearing helmet & backpack (11 kg, 12-13°C)	Ergogenic effect due to ephedrine, NOT caffeine
Bell <i>et al.</i> (2002)	15 male & 6 female Habitual & non-users Abstained 12 hours prior	5 mg/kg BW 1, 3 or 6 hours prior	TTE	↑ TTE Effect ↑ in nonusers vs. users ↓ RPE
Greer <i>et al.</i> (2000)	8 males Abstained 48 hours prior	6 mg/kg 90 minutes prior	Cycle TTE	↑ TTE

Reference	Participants	Supplementation / Intervention	Outcome measured	Conclusion
Bruce <i>et al.</i> (2000)	8 male rowers Abstained 72 hours prior	0, 6, 9 mg/kg 60 minutes prior	2000 m rowing performance on an air-braked ergometer	↓ performance time ↑ mean power ↓ RPE
Anderson <i>et al.</i> (2000)	8 oarswomen Abstained 72 hours prior	6 or 9 mg/kg 60 minutes prior	2 000 m rowing trials	↓ performance time (both dosages) RPE not ↓
Van Soeren <i>et al.</i> (1998)	6 male cyclist/runners Habitual caffeine consumers No abstinence, 2 day abstinence and 4 day abstinence	6 mg/kg 60 minutes prior	60 minute cycle TTE @ 80-85% VO ₂ max	↑ endurance NOT related to prior caffeine habituation
Kovacs <i>et al.</i> (1998)	15 male cyclists & triathletes Habitual and non-users Abstained 48 hour prior	150, 225 & 320 mg (+ CES)	60 minute TT @ 90rpm & 70% W _{max}	↑ performance
Graham <i>et al.</i> (1998)	8 male, 1 female runner Habitual and non-users Abstained 48 hours prior	4.45 mg/kg 60 minutes prior	Running @ 85% VO ₂ max until voluntary exhaustion (~32 minutes)	↑ endurance with caffeine
Denadai <i>et al.</i> (1998)	8 males Non-users	5 mg/kg 60 minutes prior	Cycle TTE (10% above and below AT)	10% below AT: ↓ RPE , ↑ TTE 10% above AT: no effect on RPE / TTE
Bell <i>et al.</i> (1998)	8 males Habitual caffeine users	5 mg/kg 60 minutes prior	TTE during high intensity cycling RPE	↑ TTE (caffeine + ephedrine) C / E alone no effect on TTE ↓ RPE
Cole <i>et al.</i> (1996)	10 male runners & triathletes Habitual and non-users Abstained 48 hours prior	6 mg/kg 60 minutes prior	30 minutes of isokinetic variable-resistance cycling exercise	Ergogenic role identified
Cohen <i>et al.</i> (1996)	5 male, 2 female runners	5 & 9 mg/kg	21 km during heat & humidity	No significant effect on performance
Trice <i>et al.</i> (1995)	8 male cyclists	5 mg/kg 60 minutes prior	Intermittent cycling at high intensity & cycling TTE	↑ TTE
Pasman <i>et al.</i> (1995)	9 cyclists Habitual and non-users Abstained 3 days prior	5, 9 & 13 mg/kg	Cycling TTE @ 80% W _{max}	↑ performance when compared to placebo No difference at different dosages
MacIntosh <i>et al.</i> (1995)	7 male & 4 female swimmers Habitual and non-users Abstained 48 hours prior	6 mg/kg 2½ hours prior	1500 m swim (~ 20-25 minutes)	↓ RPE for 100 m warm-up swims ↓ time to swim 1500m
Graham <i>et al.</i> (1995)	8 male runners Habitual and non-users Abstained 48h prior	3, 6 or 9 mg/kg 60 minutes prior	Running at 85% VO ₂ max to voluntary exhaustion	↑ endurance with 3 & 6 mg/kg No significant effect with 9 mg/kg

Reference	Participants	Supplementation / Intervention	Outcome measured	Conclusion
Graham <i>et al.</i> (1991)	1 female & 6 male runners Habitual and non-users Abstained 48 hours prior	9 mg/kg 60 minutes prior	Running & cycling TTE @ 85% VO ₂ max	↑ TTE during running & cycling
French <i>et al.</i> (1991)	6 male runners Non-users	10 mg/kg Immediately prior	Treadmill run @ 75% VO ₂ max for 45 minutes, speed increased and TTE measured	↑ distance covered ↑ lactate (final measurement) TG ↑
Dodd <i>et al.</i> (1991)	17 moderately trained males Habitual & nonusers	0.3 & 5 mg/kg	Cycling TTE	No ↑ TTE
Rodrigues <i>et al.</i> (1990)	6 male runners	5 mg/kg	Incremental cycling TTE	No effect on TTE ↓ RPE
Gastin <i>et al.</i> (1990)	8 runners Non-users	5 mg/kg 3.5 hours prior	Incremental treadmill running	Running TTE, VO ₂ max & lactate threshold unaltered ↓ RPE
Flinn <i>et al.</i> (1990)	9 male cyclists Non-users	10 mg/kg 3 hours prior	TTE	↑ TTE
Tarnopolsky <i>et al.</i> (1989)	6 runners Habitual caffeine consumers	6 mg/kg 60 minutes prior	90 minutes treadmill running @ 70% VO ₂ max	↑ FFA, but no metabolic / neuromuscular effect that would prove ergogenic ↓ RPE
Falk <i>et al.</i> (1989)	23 males	5 mg/kg & again 2 doses of 2.5 mg/kg during exercise	Following a 40 km march, subjects completed cycle TTE protocol	No difference in TTE / RPE ↑ lactate
Fisher <i>et al.</i> (1986)	6 females Habitual caffeine users Abstained 4 days prior	5 mg/kg	60 minutes @ 75% VO ₂ max treadmill	Caffeine after withdrawal ↑ effects
Powers <i>et al.</i> (1983)	7 male cyclists Abstaining 2 weeks prior	5 mg/kg 60 minutes prior	Graded cycle ergometer exercise, TTE	No significant difference in the TTE
Ivy <i>et al.</i> (1979)	7 male & 2 female cyclists Abstained 12 hours prior	250 mg 60 minutes prior Repeated doses of 250 mg every 15 minutes for the first 90 minutes	120 minutes of isokinetic cycling exercise @ 80 rpm	↑ work production RPE unchanged
Costill <i>et al.</i> (1978)	7 male & 2 female cyclists Abstained 6-12 hour prior	330 mg caffeine in coffee 60 minutes prior	Cycle TTE @ 80% VO ₂ max	↑ TTE ↓ RPE
Perkins <i>et al.</i> (1975)	14 female students	0, 4, 7 & 10 mg/kg	Incremental cycling TTE	No effect on performance or RPE

Sources: (13-15, 19-22, 25-27, 29, 30, 32, 37, 45, 53, 75-118)

RPE: Rating of perceived exertion; TTE: Time to exhaustion; TT: Time Trial; mg/kg: milligrams per kilogram body weight; CES: Carbohydrate-electrolyte solution; HR: Heart rate; RER: Respiratory exchange ratio; FFA: Free fatty acids; kJ/kg: kilojoules per kilogram body weight; AT: Anaerobic threshold

By analysing the abovementioned studies in Table 2.1, it appears that caffeine supplementation decreases the time to complete time trial events, increases endurance when exercising to exhaustion (i.e. increases time to fatigue), decreases ratings of perceived exertion and increases prolonged sub-maximal cycling performance. This is also evident when reading earlier reviews on the beneficial effect of caffeine supplementation on decreasing time to exhaustion during endurance testing (28) and reducing the rating of perceived exertion (31).

There appears to be four performance indicators often used when studying the ergogenic effect of caffeine supplementation (Table 2.1). These indicators include endurance time to exhaustion, steady-state exercise, ratings of perceived exertion and time trial performance. From Table 2.1 one can see that most studies were completed using time to exhaustion or steady state exercise trials

Only a few studies have found a beneficial effect of caffeine supplementation on increased exercise performance following time trial protocols. These include studies examining the effect of caffeine supplementation on i) 8 km running (13), ii) 90 minutes cycling at 65% $\text{VO}_{2\text{max}}$ followed by a 15 minute time trial (78), iii) 60 minute cycling (16, 25), iv) 2000 m rowing (14, 102) and v) 1500 m swim (15).

Previous studies on caffeine supplementation involved subjects abstaining from caffeine for periods of between 2-4 days. The general consensus is that the dosage of caffeine supplementation needed to result in an ergogenic effect is 3-13 mg/kg body weight (69) (Table 2.1). Lower dosages (3 mg/kg body weight) also appear to be ergogenic (69). It is also suggested that the timing of caffeine ingestion (before and then repeatedly during exercise) is not of paramount importance, provided that the initial dose of caffeine is sufficient to maintain optimal plasma levels for the duration of the exercise (27, 98, 100). These abovementioned studies evaluating caffeine supplementation on time trial performance are standardized in terms of utilizing a time trial protocol as well as dose of caffeine supplementation (3-6 mg/kg BW), timing of caffeine supplementation (60-120 minutes prior to exercise) and abstinence from caffeine (24-72 hours).

There is also a large amount of studies demonstrating decreased RPE following caffeine supplementation (27, 29, 81, 91, 92, 96, 97, 99, 104, 109, 110, 112, 117, 118). This decrease in RPE does not necessarily always translate to an increased exercise performance (91, 92, 99, 109, 110), however various studies have found an increase in exercise performance without observing decreased RPE (75, 77, 83, 85, 102).

The majority of the studies regarding exercise and caffeine supplementation employ TTE exercise protocols. Although these studies show an increased TTE with caffeine supplementation and therefore promise of its ergogenicity, it is limited in terms of practical applicability of the results obtained compared to the field setting. There is a greater coefficient of variation when a subject performs a TTE protocol in comparison to time trial performance (41-43). Time to exhaustion does not measure the true exercise performance benefit of caffeine as no sport requires an athlete to endure more or complete a longer distance than his / her competitors (41, 42). Another limitation is that several studies (28-30) also use protocols in which athletes exercise for longer periods of time (a total of 120-240 minutes) at constant sub-maximal intensity (steady state), which is not applicable to a race situation.

Studies that found that caffeine supplementation did not have a beneficial effect on sports performance were performed using non-endurance exercise tests or events. These studies investigated the following aspects: i) athletic agility (35), ii) weight training (36), iii) 10 km running performance while wearing a helmet and backpack weighing 11 kg in a climate suite during environmental conditions of 12 – 13°C (37), iv) repeated sprints in team-sport athletes (38), v) maximal ability to generate power or reduction in fatigue during high-intensity dynamic exercise (39), or vi) repeated bouts of short term intense exercise measured by Wingate tests (40).

Other studies (45, 106, 110, 119) that also showed little or no beneficial effect of caffeine supplementation were not standardised in terms of nutrition, environmental factors, or the type of exercise performed. For example, the time to exhaustion during high-intensity cycling was measured in non-athletic males consuming caffeine on a habitual basis, but caffeine intake prior to the test was not standardised (45), endurance racing during hot and humid conditions (106), activities requiring strength and short-term endurance (119), and incremental treadmill running (110).

The addition of other dietary components, such as CHO, which is common practice during endurance exercise, negates some of the ergogenic effects of caffeine, as shown in a review on the effect of the ingestion of CHO and caffeine compared to the effect of caffeine intake alone. Caffeine intake increased performance, irrespective of CHO intake; however, the magnitude of difference was smaller when combining caffeine with CHO (120).

It thus seems that caffeine has a clear benefit in enhancing exercise performance in steady-state endurance exercise. During a triathlon, however, exercise can be stochastic at times,

making it difficult to extrapolate research findings from the abovementioned studies to a triathlon.

Although studies employ triathletes as part of the study population, it is evident from the literature explored that the discipline of triathlons is not commonly researched, especially in terms of caffeine supplementation. This is possibly due to the complexity of the sport as a model. Triathlon encompasses three sporting disciplines namely swimming, cycling and running. An Olympic or standard distance triathlon comprises of a sequential swim (1.5 km), swim-to-cycle transition, cycle (40 km), cycle-to-run transition and run (10 km) (2, 8). The time spent completing an Olympic-distance triathlon can range from 1 hour 50 minutes for professional male athletes, to slightly over 2 hours for professional female athletes and 2 hours 20 minutes for amateur-level male and female triathletes (121).

Studies completed on endurance athletes, using protocols including swimming, cycling and running, can be indicative of triathlon performance to a certain degree. However, this is not always ideal, as the physiological requirements of a triathlete differs significantly from that of an athlete competing in only one discipline, due to long-term training conditioning. Triathletes exhibit different physical and physiological characteristics, which may enable them to perform better in different environmental conditions and landscapes (2). For example, a host of environmental circumstances, the use of a wetsuit, drafting during swimming and cycling, power output and cycling cadence selection, all potentially increase the functional demand of a triathlon (47, 48). The variety of event distances available for triathletes to compete in also leads to differences in training programs; and technical, physiological and nutritional differences, depending on the distance of the triathlon and should be taken into consideration (122).

There is therefore a need for sport-specific studies to be conducted that investigate the use of potential performance-enhancing supplements, such as caffeine, particularly in semi-elite or provincial level athletes.

2.3 Mechanism(s) of action of caffeine

The mechanism of action of caffeine in order to produce an ergogenic effect can be explained by the pharmacodynamic properties of caffeine. The pharmacodynamics of a drug considers the concentration-effect relationship and thereby explaining the effect that the drug has on the body (3).

In vitro studies have helped to elucidate a multitude of cellular actions of caffeine. Although these effects are not always significant *in vivo*, it does appear that these various possible cellular actions of caffeine contribute to its observed effects (64). The paucity of research on the mechanism of action of caffeine and the controversy surrounding this has also been documented and many authors ascribe the ergogenic properties of caffeine to more than one mechanism (51, 54, 57, 101, 123-126).

Caffeine has numerous effects on the body, including effects on the central nervous system (CNS); and on metabolic, hormonal, cardiovascular, pulmonary and renal functioning during rest and exercise (69) (Figure 2.1). These effects can lower the respiratory exchange ratio, peripheral fatigue, rating of perceived exertion (RPE), and the threshold for exercise-induced cortisol and beta-endorphin release; caffeine can increase oxygen uptake, cardiac output, ventilation, circulating levels of epinephrine, metabolic rate and fat oxidation during endurance exercise in trained and untrained subjects (69).

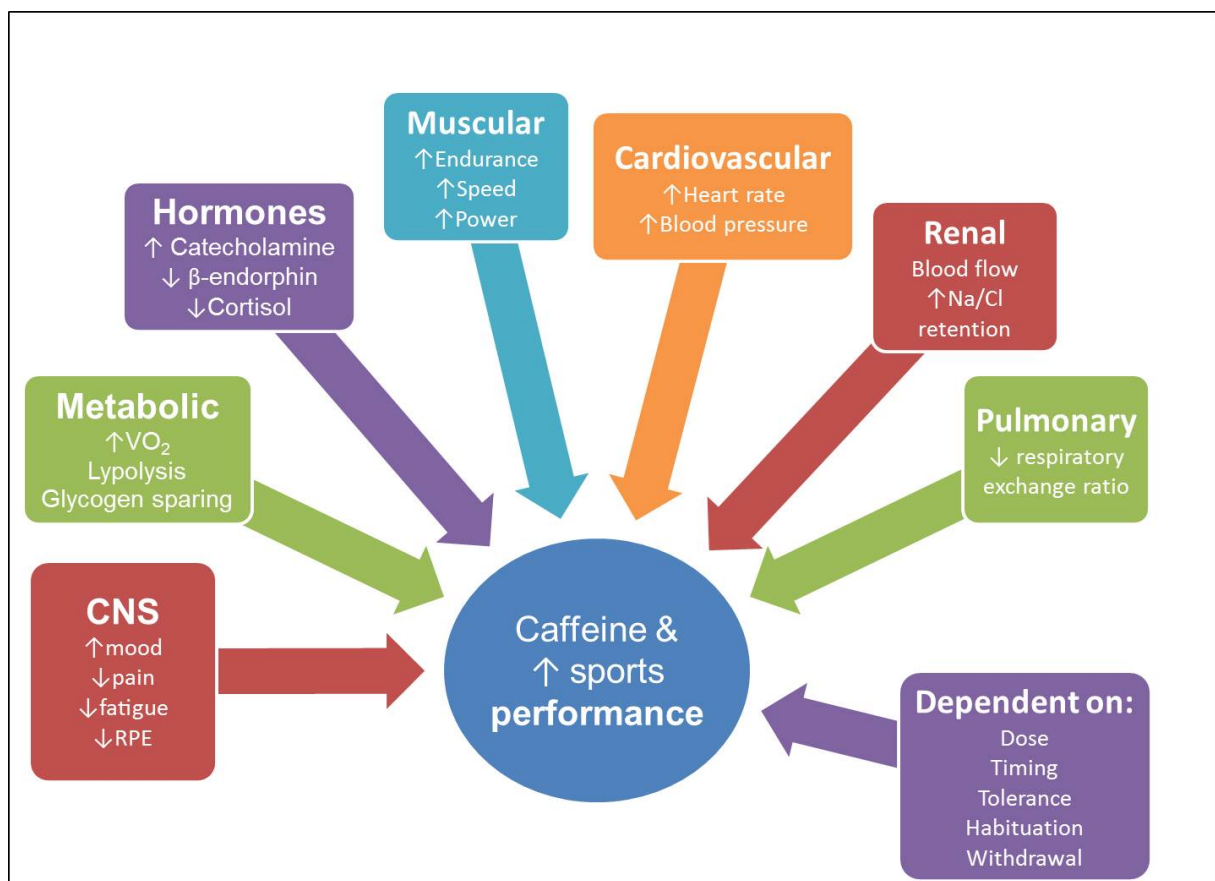


Figure 2.1 The effects of caffeine on body systems and sports performance

Source: (69)

Numerous methods of action of caffeine have been reported. A systematic review of the effect of caffeine on sport-specific endurance performance proposed three mechanisms for

the ergogenic effect of caffeine, namely (41): i) Enhanced mobilization of intracellular calcium; ii) an increase in free fatty acid oxidation; and iii) antagonism of adenosine receptors in the CNS. An additional mechanism of action of the ergogenic effect of caffeine that has been proposed is the inhibition of cyclic nucleotide phosphodiesterases (57). This involves inhibition of cyclic nucleotide breakdown *via* the inhibition of phosphodiesterase and high quantities of caffeine is needed to elicit this effect (20 times higher concentrations) (57).

Dated and more recent reviews by Goldstein *et al.* (2010), Tarnopolsky (2010), Davis *et al.* (2009), Ganio *et al.* (2009), Sokmen *et al.* (2008), Jones (2008), Keisler *et al.* (2006), Kalmar *et al.* (2004), Graham *et al.* (2001), Sinclair *et al.* (2000), Spriet (1995) and Clarkson (1993) have been published with detailed information on the mechanisms of action of caffeine in relation to exercise performance (41, 50, 51, 54, 69, 72, 74, 123, 127-130). Figure 2.2 illustrates the most important of these *in vivo* mechanisms with regard to sport performance. The section thereafter highlights these mechanisms with regard to the biophysiological rationale for investigating these mechanisms in terms of sport performance.

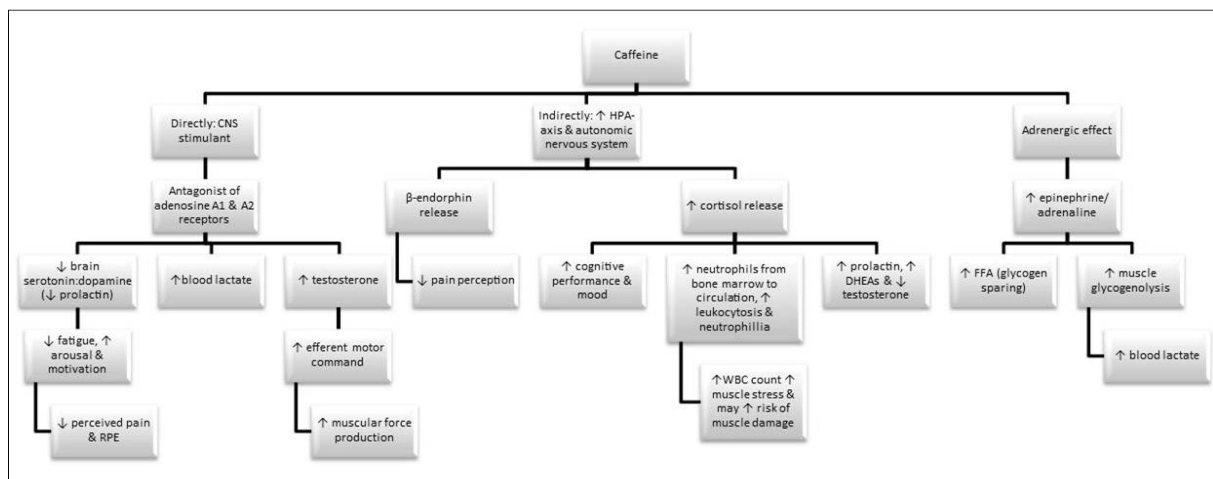


Figure 2.2 In vivo mechanisms of action of caffeine with regard to sport performance

Sources: (52, 53, 131-134)

2.3.1 The direct effect of caffeine on the central nervous system

It is evident from the literature that the most profound effect of caffeine supplementation on performance is as a result of direct stimulation of the CNS (72) where caffeine acts as an antagonist of adenosine receptors (41, 50, 51, 54, 69, 73, 74, 123, 128-130), particularly adenosine receptors A_1 and A_2 . This receptor antagonism results in delayed fatigue, increased arousal, motivation, wakefulness, alertness and vigilance (123, 130). There are multiple actions resulting from the direct effect of caffeine on the CNS and the antagonism of adenosine receptors. These include a decreased brain serotonin:dopamine ratio (and

consequently a decrease in prolactin levels), increased testosterone and blood lactate levels, as well as leukocytosis. The implication of which will now be briefly discussed.

The antagonism of the adenosine receptors leads to a decreased serotonin:dopamine ratio in the brain (53). Increased levels of serotonin are found during prolonged exercise and leads to increased fatigue (central fatigue hypothesis). The release of the hormone, prolactin is under control of the central serotonergic system and is responsible for increasing serotonin synthesis and decreasing levels of dopamine. Caffeine decreases serotonin and increases dopamine, leading to a favourable serotonin:dopamine ratio. Thus, if caffeine acts by means of improving the brain serotonin:dopamine ratio and thereby reducing fatigue, increasing arousal, motivation and decreasing perceived pain and exertion, prolactin levels could be reduced in exercise trials examining the effect of caffeine supplementation (53).

The abovementioned effect of caffeine on the adenosine receptors of the CNS thus leads to a decrease in perceived pain and decreased ratings of perceived exertion (41, 54, 74, 127, 130) by binding to receptors (A_1 and A_{2A}) and decreasing neurotransmitters (69, 123). This action leads to reduced tiredness, improved mental alertness, mood and energy (69).

In addition to the effect of caffeine on prolactin, caffeine may increase testosterone levels. Although the exact mechanism by which caffeine supplementation can increase testosterone levels is unclear (134), it can be hypothesized that because caffeine antagonizes the adenosine receptors in the CNS, it facilitates an increase in muscular work output. An increase in testosterone is linked to the intensity of exercise and increases with an increased exercise intensity. Therefore, if caffeine can further increase work output, testosterone levels would be further elevated (134). However, the elevated testosterone might be counteracted by an increased release of cortisol as discussed in section 2.3.2.

Caffeine also increases blood lactate levels during endurance exercise, primarily due to increased levels of epinephrine as discussed in section 2.3.3 or due to stimulation of the CNS (51, 130). Caffeine has been shown to increase blood lactate levels (51), particularly during endurance exercise (130). This has been observed more frequently than increases in the levels of FFA, or decreases in respiratory exchange ratio's (51). Increased lactic acid levels have also been observed at rest following caffeine ingestion (130).

Davis, in a review published in 2009 found enhanced exercise performance with caffeine supplementation, irrespective of whether lactate levels had increased or not. The author speculated that the reason for the increased lactate levels may be that caffeine stimulates

either the release of epinephrine or the CNS and thereby reducing pain. The reduced pain sensation can also increase lactate accumulation and therefore the two factors can also be present as a coincidence (130).

A further result of the antagonism of the adenosine receptors by which caffeine has its affect on the body can be seen on circulating neutrophils. This action is most likely also due to the antagonism of the adenosine receptors, which leads to an increase in the neutrophil oxidative burst response. An effect on neutrophils may also be present due to the adrenergic effect of caffeine (Section 2.3.3), although this would result in a detrimental effect on the neutrophil response (132, 133). Plasma adenosine is increased during exercise due to the dephosphorylation of AMP. Neutrophils express A₁ and A₂ adenosine receptors on their surfaces. Adenosine acting via A₁ receptors promotes neutrophil chemotaxis and phagocytosis and adenosine acting via A₂ receptors inhibit neutrophil phagocytosis and superoxide generation. Caffeine is a non-selective adenosine receptor antagonist and can therefore affect positive and negative aspects of neutrophil function (132, 133). Neutrophils form 50-60% of circulating white blood cells and are important for the release of reactive oxygen species and antimicrobial enzymes. It provides the first line of defence against infectious agents and is typically reduced during or after prolonged, intensive exercise. The neutrophil oxidative burst response is the capacity of neutrophils to generate reactive oxygen species and caffeine has been shown to attenuate the reduction in this response (132, 133).

2.3.2 The indirect of caffeine on the HPA-axis and autonomic nervous system

There is enhanced secretion of β -endorphins following supplementation with caffeine, which reduces pain perception during exercise (72). The resulting reduction in perception of pain can decrease RPE and improve exercise performance.

Stimulation of the HPA-axis also increases the release of cortisol. The resulting cortisol release can lead to metabolic, cardiovascular and CNS implications (54) and it increases cognitive behavior, performance and mood.

Furthermore, physical exercise is recognized as being an impetus for the release of hormones from the endocrine system in both males and females (135, 136). The release of these hormones, though, is dependent on various factors related to physical activity, such as the intensity, time, and type of exercise, as well as the individual's level of fitness (136).

The term “stress” was defined by Pruetz (2003) as “*the physiological response of the whole animal, in contrast to cellular stress responses, the induction and regulation of which are distinct from responses of the intact animal. Stimuli that induce stress responses are stressors, and common stressors include psychological, physical, and drug or chemical stimuli. Activation of this physiological response almost always is associated with activation of the hypothalamic-pituitary-adrenal (HPA) axis and the sympathetic nervous system, leading to changes in the concentrations of stress-related mediators.*” (10) The effects of such stressors on the endocrine system are illustrated in Figure 2.3.

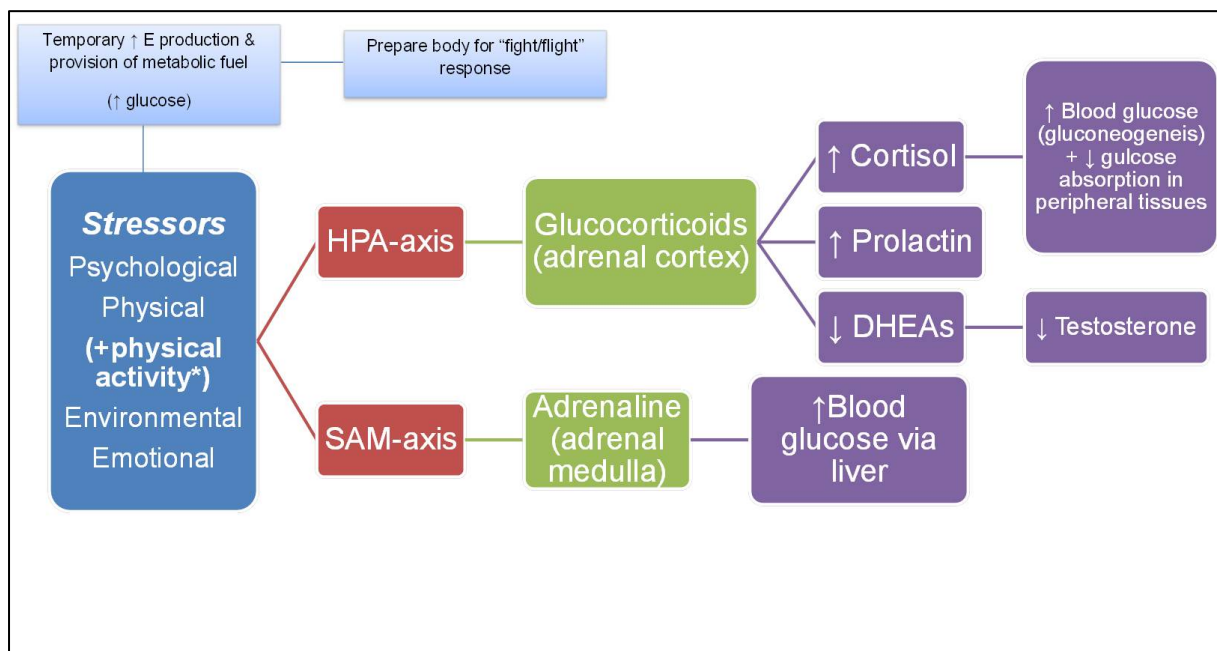


Figure 2.3 Effects of stressors on the endocrine response

Source: (10, 137)

HPA: Hypothalamic-anterior Pituitary-Adrenocortical axis; SAM: Sympatho-Adrenomedullary axis

*extent dependent on intensity, duration & tissue specificity

A stressor can be defined as a “*non-specific stimuli that disturb homeostasis and elicit an invariable stress response*” (138). The stress response stimulates the adrenal glands and lead to suppression of the immune system, mainly due to increased levels of glucocorticoids (133). The stress response includes the “fight-or-flight” reaction, which is due to acute stress, followed by the endocrine-stress response (138).

Various hormones are affected by stressors; these include cortisol, prolactin, dehydroepiandrosterone sulphate (DHEAs) and testosterone.

Cortisol

Cortisol is one of the main glucocorticoids secreted in response to stress after stimulation of the HPA axis (139). Caffeine, the mental stress of competing in a race environment and the exertion of exercising at the high intensity required during a triathlon, all increase cortisol secretion (140).

Caffeine and exercise independently elevate cortisol levels (141). Increased cortisol levels have been found after marathon running (136, 142-146) and the authors concluded that this was primarily due to stimulation of the HPA axis. The degree to which cortisol levels are elevated during or after exercise, though, is dependent on the duration of exercise (136, 145, 147). Exercise is seen as a stressor and the response of cortisol to stress varies widely between individuals (148) and between genders. Following exercise, the elevated levels of cortisol may illicit metabolic effects, such as increased blood glucose levels due to gluconeogenesis and lipolysis as shown in Figure 2.4 (141).

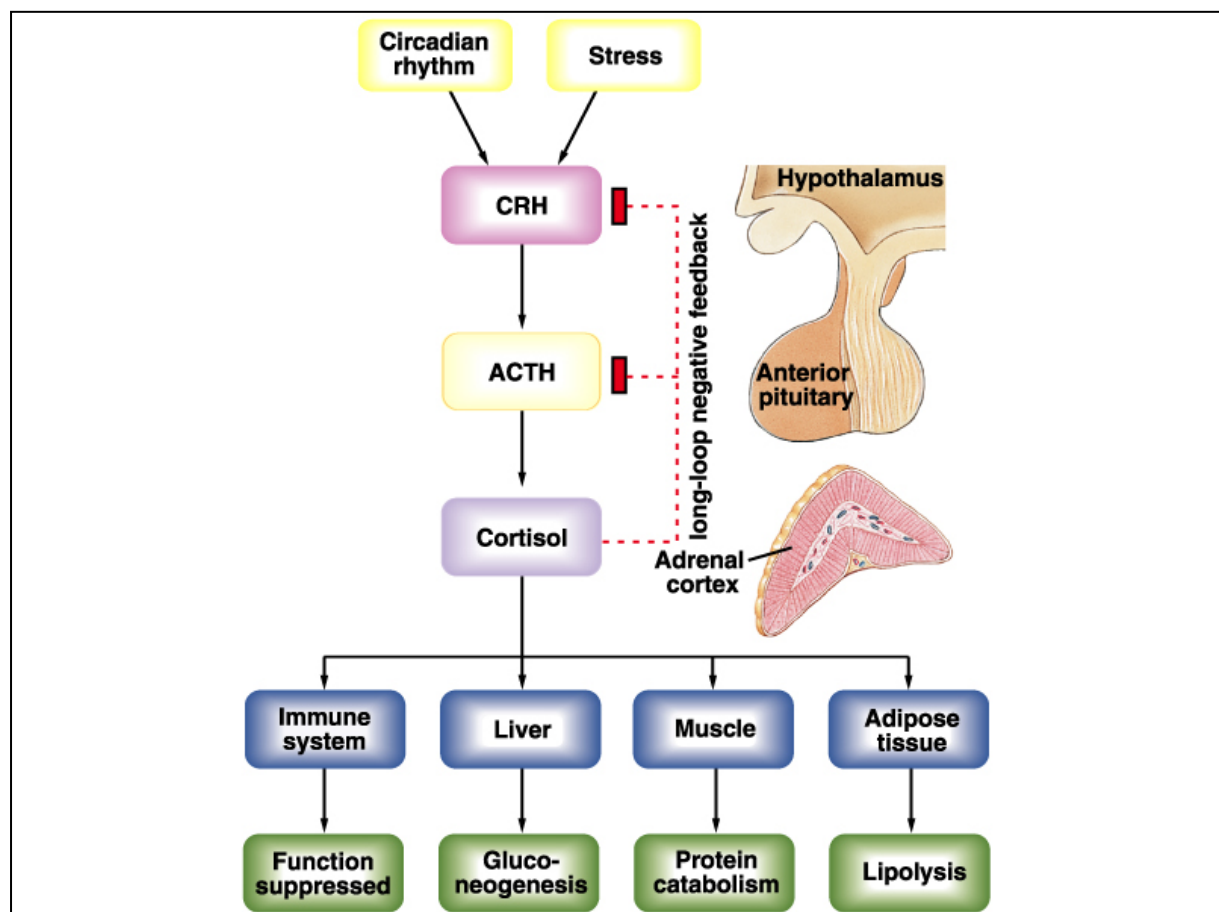


Figure 2.4 The control pathway for cortisol

Source: (5)

Lovallo *et al.* (2006) has described the main effects of caffeine and gender on the cortisol release response. Exercise and mental stress have different effects on cortisol secretion, with mental stress exerting a much more pronounced and rapid effect than exercise. Men and women also respond differently to psychological stress. Caffeine, in combination with stress, was found to result in a small, transient increase in cortisol levels in women, but lead to a substantial, prolonged increase in men. Without the introduction of caffeine, both males and females have similar cortisol responses after exercise. Caffeine thus enhances the stress response in both men and women and there are metabolic factors that can modify these relationships, especially during prolonged exercise where an increase in cortisol is present due to an increased metabolic fuel demand (140).

Prolactin

In addition to the role of neuro-modulation described in 2.3.1, prolactin also plays a role in both reproductive and other non-reproductive processes. Prolactin and growth hormone appear to be needed for T-lymphocyte differentiation in the thymus gland and prolactin levels increase, along with cortisol levels, in the presence of a variety of stressors (5, 10). Prolactin levels are known to increase in response to physical stress, such as during or after marathon running (136). Prolactin and cortisol increase together in response to stress, and the increase in prolactin levels vary depending on the intensity of the stressors. Prolactin and cortisol can both thus be useful markers of stress (149). In females, in particular, prolactin increases during prolonged exercise. This may occur to some degree as a result of increased body temperature or passive heating (135). Caffeine supplementation may have the opposite effect on prolactin compared to prolonged exercise as discussed in Section 2.3.1. The resulting decrease in prolactin due to caffeine supplementation can be favourable in terms of decreasing fatigue, increasing arousal and motivation, decreasing perceived pain and exertion and thereby improving endurance performance.

DHEAs

DHEAs play a role in the immune and stress response, and levels of this hormone increase during endurance exercise (136, 150). DHEAs is a stress-related androgen, and is a precursor required for testosterone production (135).

Androgens, such as DHEAs and testosterone, have various roles in the human body, for example in the reproductive system, in muscle growth, the prevention of bone loss, in maintaining bone density and in erythrocyte production (135). In female athletes, excessive

physical exercise can lead to a delayed onset of menarche, amenorrhea and osteoporosis, and these androgens thus become increasingly important (135). Numerous factors, including the phase of the menstrual cycle, age, nutrition, the use of oral contraceptive medication and exercise can influence androgen levels (135). DHEAs levels are increased following acute exercise in pre –and post-menopausal women, primarily due to increased release from the adrenal cortex in response to adrenocorticotrophic hormone (ACTH) stimulation (135). It is also known that women who regularly participate in endurance exercise have decreased resting DHEAs levels as a result of decreased levels ACTH following an endurance training program (135).

Testosterone

Testosterone is an anabolic hormone, mainly in the male reproductive system. Testosterone levels decrease in response to stress (10). Testosterone and cortisol have a unique interaction, as both are competitive agonists of muscular cells. The testosterone/cortisol ratio is often used as an indicator of the anabolic/catabolic balance in the body. Acute exercise, as well as long periods of training or repetitive competition can decrease this ratio. This ratio between testosterone and cortisol can indicate the actual physiological strain induced by training or exercise (151).

Studies have shown that testosterone levels increase after resistance or endurance training and decreases with prolonged endurance training, for example running a marathon. However, some studies have shown an increase in testosterone concentrations after prolonged endurance exercise (136, 150). The response of testosterone following exercise is largely dependent on exercise intensity, duration and training status (136, 146, 147). However, some studies have shown an increase in testosterone concentrations after prolonged endurance exercise (136, 150).

In females, testosterone levels are increased after prolonged endurance exercise (135) and no changes in testosterone levels are observed due to menstrual cycle changes. This increase in testosterone is mostly due to increased metabolic clearance and decreased hepatic flow during endurance exercise. Haemoconcentration due to dehydration, sweating and hydrostatic pressure that can occur during or after exercise may also play a role. It is also known that women who engage in regular physical activity, have lower baseline testosterone levels compared to sedentary counterparts (135). During resistance training, caffeine has been found to increase testosterone levels, although this effect may be

diminished by an increased release of cortisol due to the physical activity and caffeine's role as a metabolic stressor (152) as discussed in Section 2.3.1.

2.3.3 Adrenergic effect

The most controversial and widely-discussed mechanism of action of caffeine in the literature is its possible effect on substrate metabolism during exercise. It has been postulated that caffeine can decrease the body's reliance on glycogen and increase its dependence on free fatty acids (FFA) (72) as a fuel source during exercise. This proposed effect is due to the increased lipolysis of adipose tissue and intramuscular triglycerides, which results in increased levels of FFA due to the caffeine-induced release of epinephrine/adrenaline, an adrenergic effect (41, 50, 51, 54, 69, 73, 74, 127-130). The above explanation, however, lacks empirical evidence (50) and cannot explain increased performance in shorter-duration muscle fatigue protocols (50, 51, 130). It has also been noted that this glycogen-sparing effect of caffeine is overridden by the adrenergic effect of exercise (74).

Another potential mechanism that forms part of the glycogen-sparing model is that caffeine increases intracellular cyclic AMP (cAMP) levels. *In vitro* studies show that this is achieved through inhibition of phosphodiesterases (54, 128-130). Increased levels of cAMP lead to enhanced lipolysis and fatty acid oxidation, and can result in glycogen sparing (128-130). However, this mechanism is unlikely to explain the effect of caffeine on high intensity exercise (130) and is argued to not be likely in humans (74, 153).

Raised blood glucose levels occur after caffeine supplementation, due to increased epinephrine (51, 130). The release of epinephrine can reduce pain, leading to increased lactate concentrations. This increased lactate can be converted to glucose during endurance exercise, maintaining blood glucose levels and thereby decreasing the demand on liver and muscle glycogen, reducing fatigue and improving endurance performance.

The increase in epinephrine is also linked to reduced neutrophil reactive oxygen species production and it stimulates β -2 adrenoreceptors expressed by neutrophils, which leads to the activation of adenyl cyclase and inducing cAMP synthesis, inhibiting the activation of neutrophils (132, 133).

2.3.4 Other possible mechanisms

2.3.4.1 Increased intracellular calcium release from the sarcoplasmic reticulum

Caffeine increases muscular force production as a result of increased intracellular calcium concentrations, resulting in alterations in neuromuscular function and / or skeletal muscle contraction (41, 51, 54, 69, 72-74, 127-130). This positively affects exercises such as short sprints, and power performance (69). However, this effect has been described in *in vitro* models only, using levels of caffeine that are toxic to the human body (pharmaceutical concentrations needed) (50, 51, 54, 129, 130).

2.3.4.2 Altered excitation-contraction coupling

The Na^+/K^+ ATP-ase pump is important for maintaining the electrochemical gradient by transporting sodium ions (Na^+) out of cells and potassium ions (K^+) into cells (54, 129). It has been proposed that caffeine-induced increases in muscular force production occur as a result of increased Na^+/K^+ ATP-ase pump activity (54, 69, 129, 130).

Additional mechanisms of action of caffeine that have been proposed in the literature, but not fully supported include the fact that caffeine exerts a thermogenic effect by increasing energy expenditure (72) and an increase in energy status due to less phosphocreatine degradation and less accumulation of adenosine diphosphate (ADP) and monophosphate (AMP). The significance of which is questionable (51).

2.4 Factors influencing the ergogenic effect of caffeine supplementation

Various factors such as lifestyle, gender and genetics can influence the pharmacokinetics of caffeine and therefore the ergogenic effect of caffeine supplementation (Figure 2.5).

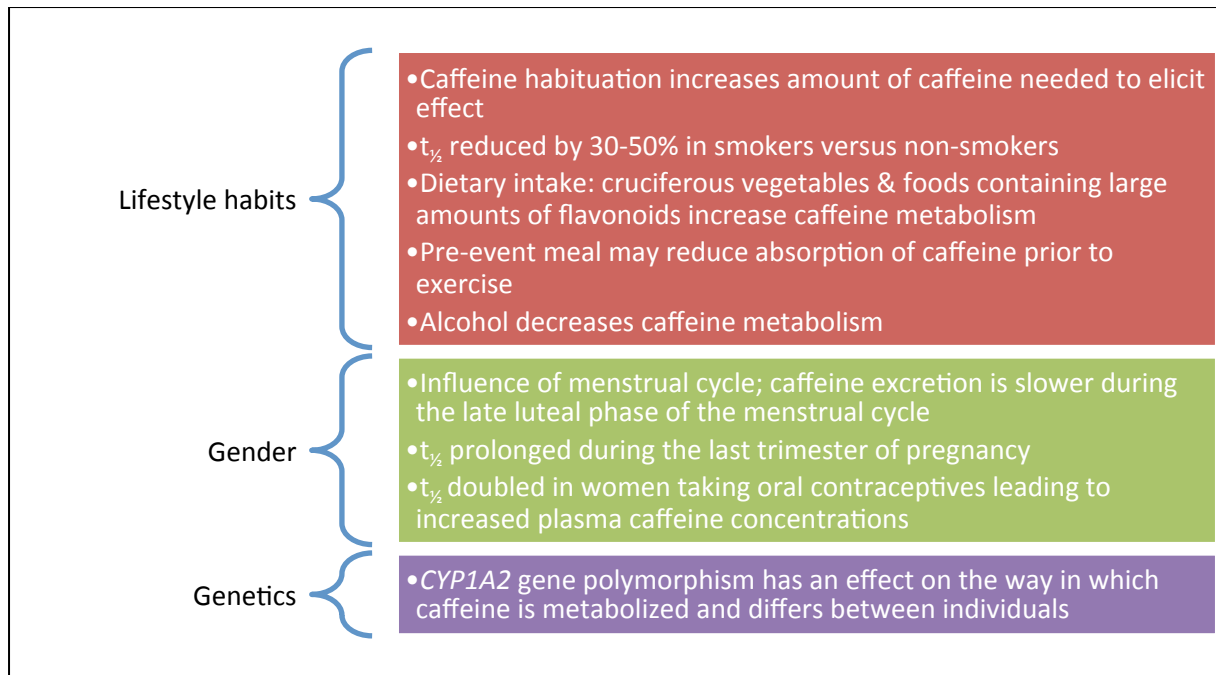


Figure 2.5 Factors influencing inter-individual differences in plasma caffeine concentrations

Sources: (54-63)

The pharmacokinetic profile of a drug considers the dose-concentration relationship and thereby describes the movement of the drug in the body. The pharmacokinetic properties of caffeine with special regard to the factors influencing the ergogenicity thereof (Figure 2.5) are discussed below.

2.4.1 Pharmacokinetic profile of caffeine

The pharmacokinetic profile of caffeine comprises the absorption, distribution, metabolism and elimination of the pharmacologically active compound (64).

2.4.1.1 Absorption

Drug absorption takes place from when the drug is administered, until it reaches the systemic circulation (3). Drug absorption can be influenced by a number of factors, such as the route of administration, type of formulation, particle size, lipid solubility, stability of the drug in an acidic environment and towards enzymatic degradation, presence of food in the stomach, drug interactions, motility of the gastro-intestinal tract and how much of the drug is subjected to first-pass metabolism (3). The timing and method of drug administration, as well as the extent to which the drug undergoes first pass metabolism. The absorption of a drug is measured by its bioavailability, which is the proportion of the dose of the drug that is administered that reaches the systemic circulation (3).

Caffeine habituation (>300 mg/day) can also lead to increased levels of caffeine needed to elicit the same effect. Repeated exposure to caffeine, or a high habitual intake, causes habituation or tolerance to develop to caffeine and can diminish the body's responsiveness to caffeine supplementation (69). Repeated intake of moderate to high amounts of caffeine can increase adenosine receptor activity as well as β -adrenergic activity, which leads to the development of tolerance within 5-6 days of moderate caffeine use (69). It is important to take habitual caffeine use into consideration (72) when seeking to enhance exercise performance by means of caffeine supplementation, as increased dosages will be needed to elicit the same effect if a subject is a habitual user (73).

The general consensus is that the dosage of caffeine supplementation needed to result in an ergogenic effect is 3-13 mg/kg body weight (69) (Table 2.1). Lower dosages (3 mg/kg body weight) also appear to be ergogenic (69). It is also suggested that the timing of caffeine ingestion (before and then repeatedly during exercise) is not of paramount importance, provided that the initial dose of caffeine is sufficient to maintain optimal plasma levels for the duration of the exercise (27, 98, 100).

It has also been found that when the initial dose and volume of caffeine are the same, the type of formulation in which the caffeine was administered (for example gum, capsules, coffee, soft drinks or chocolate) does not appear to have an effect on plasma caffeine levels (64). It has been documented that caffeine in capsule form is easy to ingest and the dosage can be controlled effectively (72). When caffeine is ingested in the form of coffee, it is difficult to attribute the true effect to caffeine and it is also difficult to establish the exact amount of caffeine found in varieties of coffee (72). During the roasting of coffee, chlorogenic acids are produced. These derivatives may influence the effect caffeine has on the CNS and thereby reducing the capacity of caffeine to inhibit adenosine receptors (154). Although the type of formulation can influence the onset of effect, and should thus be considered when determining the times of administration, the magnitude of the effect of a caffeine dose is not dependent on the type of formulation used (64).

Caffeine is absorbed quickly in the small intestine of the gastro-intestinal tract (GIT). Caffeine promotes gastric emptying by stimulating the internal myenteric and submucous nerves in the stomach. The drug is then absorbed directly from the stomach into the blood stream. Ninety nine percent of the caffeine ingested in a dose is absorbed into the blood within 30-75 minutes (57, 69, 155-157).

2.4.1.2 Distribution

Once a drug has reached the systemic circulation, it gets distributed into various compartments, such as the vascular, extracellular or intracellular fluid compartments, depending on the properties of the drug. Distribution is often described as the half-life of a drug ($t_{1/2}$), meaning the time taken for the concentration of a drug in the blood to fall by half its original value. The measurement of $t_{1/2}$ allows the calculation of the elimination rate of the drug (3).

The peak concentration of caffeine (8-10 mg/l) for doses of 5-8 mg/kg body weight is typically reached in the blood after 15-120 minutes (57, 156). There is also a direct correlation between the dose of caffeine ingested and the peak concentration in plasma for dosages of 1-10 mg/kg body weight. For dosages lower than 10 mg/kg body weight, the half-life ($t_{1/2}$) can be up to 4-5 hours (57, 69) and can be longer (6-7 hours) for absolute dosages exceeding 300 mg (69).

There are considerable inter-individual differences in plasma caffeine concentrations after caffeine ingestion (64). The extent of this variation is dependent on various factors, including gender, lifestyle habits, the type and intensity of training, genetics and the concurrent use of medication (54).

As shown in Figure 2.1, two of these factors affecting the plasma concentrations of caffeine are the phase of the menstrual cycle (i.e. whether it is the luteal or follicular phase) and the use of oral contraceptive medication. Caffeine excretion is slower during the late luteal phase of the female menstrual cycle, which occurs prior to menstruation. This increases plasma caffeine concentrations. Although this effect of the menstrual cycle is not influenced by habitual caffeine intake, the metabolism of caffeine with dosages up to 6 mg/kg body weight may be affected by the phase of menstrual cycle. (62). The use of oral contraceptives and hormone replacement therapy has also been known to interfere with caffeine metabolism and chronic use of these drugs has been found to lead to increased plasma concentrations of caffeine (63).

2.4.1.3 Metabolism

Drug metabolism occurs mainly in the liver and the aim of drug metabolism is to make the drug more hydrophilic, in order for it to be excreted by the kidneys (3). There are two main stages of hepatic drug metabolism. A phase 1 reaction is when a drug is converted into more polar metabolites. The main enzyme system involved in this is cytochrome P450. Phase II

reactions are when drugs or metabolites are still not sufficiently hydrophilic and become conjugated with endogenous compounds in order to be metabolised (3).

Caffeine is metabolized in the liver to more than 25 pharmacologically active metabolites. Caffeine metabolism takes place *via* cytochrome P450 (CYP450) mediated pathways in the liver (64). Although various CYP subfamilies are involved in the oxidation of caffeine to its metabolites, the oxidation of caffeine to its four main metabolites is catalysed predominantly by CYP1A2, the major CYP in the human liver (Figure 2.6) (158-160). The main metabolic pathway of caffeine (72-80% of ingested caffeine) is characterized by the quantitative importance of 3-methyl demethylation leading to the formation of paraxanthine (*3-N-demethylation*) (57, 159). This metabolism of caffeine to paraxanthine is catalysed by CYP1A2 and can thus be used to phenotype individuals with regard to CYP1A2 (57).

Due to genetic and environmental factors, there is a large inter-individual variation in CYP1A2 enzyme activity (161). *CYP1A2* gene polymorphism and epigenetic factors (DNA methylation), as well as ethnicity and gender have been reported to affect the way in which caffeine is metabolized (161, 162). Environmental factors such as smoking, use of oral contraceptive medication, habitual caffeine intake (>3 cups per day increases enzyme activity), the use of medication (proton-pump inhibitors, antimalarial drugs, drugs given for schizophrenia) and dietary factors (intake of cruciferous vegetables and char-broiled meat) can also influence CYP1A2 activity (162). Due to these inhibitory/inductor factors, a large variability in the metabolism of caffeine exists (160).

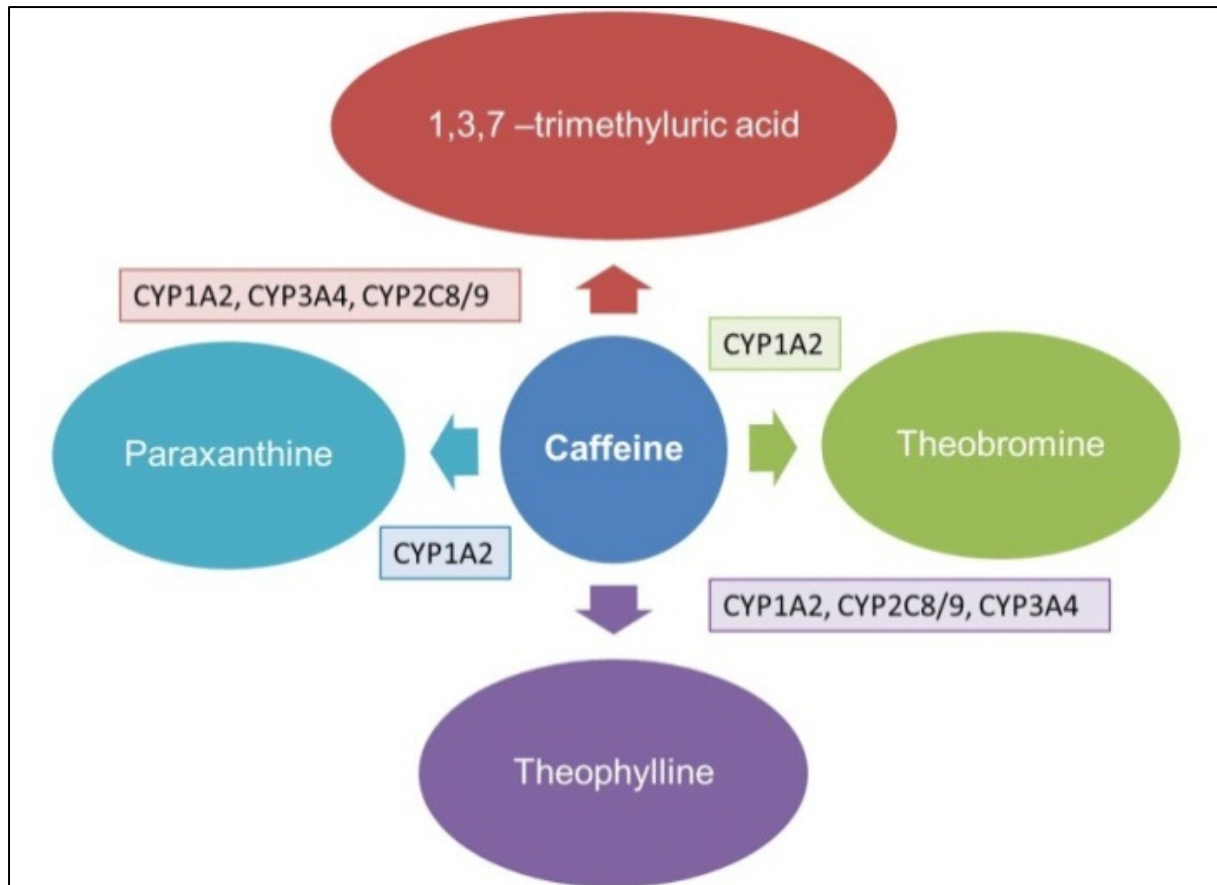


Figure 2.6 Main metabolic pathways of caffeine and the contribution of P450 isoforms
Source: (159)

The *CYP1A* gene cluster is mapped to chromosome 15 and there is a close link between *CYP1A1* and *CYP1A2*, sharing a common 5'-flanking region. *CYP1A2* is primarily regulated by the aromatic hydrocarbon receptor (AhR) (160). The *CYP1A2* gene spans 7.8 kb, comprising of seven exons and six introns. More than 20 variant alleles and sub variants of the *CYP1A2* gene have been found (<http://www.cypalleles.ki.se/cyp1a2.htm>) and five variant alleles have been shown in the literature to be associated with altered caffeine metabolism, i.e. *CYP1A2**1F (rs762551) and *CYP1A2**1D (rs35694136) (160), variants between *CYP1A1* and *CYP1A2* (rs2472297-T (163), and rs2470893-A, (164)), and the variant near the *AHR* gene (rs698865-T (163)).

Of these, only the effect of *CYP1A2**1F (rs762551) has been studied with regards to its effect on caffeine supplementation in terms of sport performance (165). Womack *et al.* (2012) investigated the effect of this specific polymorphism on the performance enhancing effect of 6 mg/kg body weight caffeine supplementation in 35 well trained male cyclists. The authors found that the 40 km cycle time was reduced by almost 4 minutes in the A/A homozygotes vs. only ~1 minute reduction in the C-allele carriers (165). This is in keeping with literature on this single nucleotide polymorphism (SNP), indicating that carriers of the C-

allele has an increased risk for developing heart disease due to slower caffeine metabolism (158, 165). Carriers of the C-allele are “slower” metabolizers of caffeine and therefore have increased levels of plasma caffeine after the ingestion thereof compared to individuals who are homozygous for the CYP1A2*1F-allele, who are “rapid” metabolizers of caffeine (158). The increased plasma caffeine levels may increase the chances of experiencing side-effects associated with caffeine ingestion, such as nervousness, restlessness, shakiness, anxiety, heart palpitations, flushing, sleep alteration, headaches and gastro-intestinal disturbances and ultimately negatively affect general health and exercise performance.

2.4.1.4 Elimination

The elimination of a drug and / or its metabolites refers to getting the drug and/or metabolite out of the body. Elimination mainly takes place via the kidneys, through urine, but can also take place through bile into the small intestine, sweat, faeces and exhalation (3).

Caffeine is reported to have a half-life ($t_{1/2}$) ranging between 2.5-10 hours. The $t_{1/2}$ is dose dependent, with higher caffeine dosages and repeated dosages increasing $t_{1/2}$ (64). This, however, is only true for dosages up to 10 mg/kg as saturation of plasma caffeine levels occur when plasma caffeine concentrations approach 100 μ M (64). Caffeine is mainly excreted by the kidneys via urine. Five percent of an administered dose of caffeine is eliminated in the urine (155 ml/kg/hour) (57). This is favourable in terms of sport, as previous guidelines included restricting caffeine use in sport to urinary caffeine levels below 12 μ g/l. Dosages of up to 9 mg/kg body weight can be administered without reaching these urinary caffeine levels (64).

Caffeine can be a mild diuretic; however, the effect of caffeine on rennin is usually only seen a few hours (approximately 4 hours) after ingestion. In the case of a sporting event lasting between two and two and a half hours, the diuretic effect of caffeine will be minimal. This is because the exercise occurs and overrides the potential for diuresis, due to an increased release of catecholamines and the renin-angiotensin-aldosterone cascade increasing solute reabsorption and improving water retention by the kidneys.

Studies that investigated the loss of body mass, sweat rates, plasma volume and electrolytes, and core temperature found that caffeine ingestion did not influence these parameters for between 1-4 hours (51). A review by Graham *et al.* (2001) concluded that “*there does not appear to be any basis for the common concern that caffeine ingestion will dehydrate athletes*”. Even though a mild diuresis can be present, literature indicates that this

does not have any measurable effect on plasma volume, sweat rate or plasma or urine osmolality (51, 72, 74). Reviews by Armstrong (2002 and 2007) also concluded that caffeine ingestion before or during exercise will not lead to fluid –and electrolyte imbalances or impair exercise-heat tolerance (166, 167).

2.5 Factors influencing triathlon performance

In order to establish whether improved performance results from caffeine supplementation, it is important to also consider confounding factors influencing triathlon performance.

These factors include general health, dietary intake two days before and dietary strategies followed on race day, body composition and bone mineral density, training or tapering before the event, withdrawal symptoms of caffeine abstinence, side-effects of caffeine supplementation and hydration status. A brief overview of the effect of dietary intake, body composition and bone mineral density on endurance performance is provided below. The remainder of the factors are described in Chapter 3 as part of the methodology.

2.5.1 Dietary intake

The importance of sound scientific guidelines on the amount and timing of food intake in relation to exercise cannot be disputed. Nutrition compliments exercise programs, assists athletes in training more effectively, and decreases the risk of becoming ill or injured. A variety of foods should be included in a typical diet; there should be ample amounts of CHO, protein, fat and micronutrients. For most athletes, a well-balanced diet is sufficient to meet their energy, macro- and micronutrient requirements. This was supported by results of a previous study examining the habitual dietary intake of a group of provincial-level triathletes in the Western Cape (71). The triathletes in this study reached most of their daily dietary requirements through food intake.

2.5.1.1 Energy and energy availability

In this specific study by the researcher (71), the body composition, dietary intake and supplement use of 26 triathletes residing in the Western Cape was studied. The average habitual dietary energy intake for men and women was 14535 kJ and 9004 kJ respectively. The CHO (CHO) intake was 5.3 g/kg body weight for males and 3.5 g/kg body weight for females. Protein intake was at the upper end of requirements for males at 2 g/kg body weight and 1.2 g/kg body weight for females. Fat intake ranged from 30-35% in males and females. We concluded from this study that overall habitual dietary intake was adequate. In

specific groups where the CHO intake was insufficient, this was attenuated by the majority of these athletes using CHO supplements (71).

In athletes with a high body mass and those participating in extreme high volume, intense training, it might prove more difficult to meet dietary requirements (168-170), especially as high intensity training can easily decrease an athlete's appetite (4, 170, 171). Endurance athletes in particular have been known to have a negative energy balance. This is primarily because body composition and factors such as gastrointestinal comfort play an important role in athletic performance (4, 170).

In order for endurance athletes to reach dietary requirements, without additional supplementation, care should be taken to eat 4-6 meals per day, including energy-dense sources of macro –and micronutrients (170, 172). According to the latest International Olympic Committee (IOC) consensus conference on sport nutrition, an important part of managing an athlete's diet includes the calculation of estimated energy availability ($_{est}EA$). Estimated energy availability is defined as “dietary energy intake minus energy expended in exercise ($_{est}EA = EI - EEE$) and is expressed in kcal/kg fat free mass (FFM)” (4). Endurance athletes are at an increased risk to develop an energy deficit and thus have a low $_{est}EA$. The $_{est}EA$ of 41 studies on endurance athletes were retrospectively calculated and ranges between 15-45 kcal/kg FFM was found amongst amenorrheic and eumenorrheic female runners, while male runners had $_{est}EAs$ between 40-45 kcal/kg FFM (173). Possible reasons given by the authors for this low $_{est}EA$, especially in female endurance athletes, include optimizing body composition, prevalence of eating disorders, having no “biological drive to match energy intake to activity-induced energy expenditure” and therefore establishing that appetite is not an accurate indicator of energy requirements, especially in endurance athletes (173). Furthermore, other factors not pertaining to the sport itself, such as pressure from media and the perception of being overweight, even when the athlete is clearly not overweight was also possible contributors (173).

2.5.1.2 Carbohydrate

The frequency and timing of CHO intake is important in physically active individuals. Optimizing pre, during- and post-workout nutrition is an essential complimentary factor to any training program. If this is not possible, it is imperative to ensure sufficient intake of CHO during the day (168). Muscle glycogen and blood glucose are the main sources of energy for contracting muscles. Optimal CHO intake assists endurance athletes specifically with refuelling glycogen stores post-exercise. This may be difficult in some instances, especially

when including high-fibre CHO (168, 170, 174). The body has restricted glycogen stores, and when these are full, they only last for between 90 – 180 minutes during moderate and high intensity exercise (174). Loading with CHO prior to an endurance event thus optimizes glycogen stores and is essential for exercise lasting longer than 90 minutes (168). This is important in order to sustain work output and increase performance.

This regime of fuelling glycogen stores in the days leading up to a race or competition is complimented by eating a high CHO pre-event meal before the competition (168, 169). For endurance events lasting longer than 90 minutes, it is essential to include CHO intake during the event. Common complaints during endurance events include muscle fatigue and hypoglycaemia, often as a result of decreased muscle glycogen stores. The type, amount and timing of CHO intake during exercise are important factors to consider and should be tailored according to individual preferences. As mentioned above, CHO intake is primarily responsible for filling the body's glycogen stores. Adequate amounts of CHO taken after exercise are also important, to increase muscle glycogen synthesis and therefore refuelling. This is especially pertinent when there is less than 8 hours' recovery time between events or training sessions (168, 169).

2.5.1.3 Protein

Dietary protein requirements are increased with exercise, albeit strength, speed or endurance training. Other factors, such as total energy intake, exercise intensity and duration, ambient temperature, gender and age also influence protein requirements (175). Protein requirements are slightly elevated in endurance exercises in particular, compared to the requirements of sedentary individuals, due to the increased oxidation of leucine, a branched chain amino acid, during endurance exercise (175). The average protein requirement for a sedentary person, according to the Dietary Reference Intakes (DRIs) and more specifically the Recommended Dietary Allowance (RDA) is 0.8 g/kg body weight/day (176). For general fitness this requirement would suffice and can be slightly elevated to 1.0 g/kg body weight / day. As seen in Potgieter *et al.* (2011), protein requirements are easily met when energy intake is sufficient (71).

Timing the protein intake in relation to training sessions can also provide more benefit in terms of recovery and improved metabolic adaptations to training (175). The ISSN recommends that, depending on the individual's exercise duration and fitness level, protein should be included with CHO in the pre-event meal (174). The addition of protein to CHO (at a CHO: protein ratio of 3-4:1) during exercise has shown some promise in recent literature.

For example, it has proven favourable in terms of increasing endurance performance and, increasing muscle glycogen stores, reducing muscle damage and promoting better training adaptations after resistance training (174, 177-180). Whether this addition of protein is due to the protein or due to the increased energy from the protein added to the supplement is still to be determined. There are studies showing no improvement in performance with the addition of protein (181, 182). Although recommended by the ISSN to add protein to CHO during endurance exercise, there is still insufficient evidence to unequivocally support this practice (175, 183).

2.5.1.4 Fat

The dietary fat recommendations of physically active individuals are mostly similar, slightly higher than their sedentary counterparts. Sufficient fat in the diet is essential to achieve optimal health and well-being, maintaining energy balance and the meeting requirements for essential fatty acids and the fat-soluble vitamins A, D, E and K. The importance of replacing intramuscular triacylglycerol (IMTAG) stores should not be overlooked in the context of sport performance. The fat requirements depend mostly on the athlete's training program and goals for nutrition support. Moderate intake of fat (30% of total energy) is required, but this can be elevated to 50% of total energy for extreme training programs or athletes with a large body mass (170, 184).

2.5.1.5 Micronutrients

Essential micronutrients are important in terms of overall health and well-being. It is unclear whether most vitamins and minerals demonstrate an ergogenic effect, and further research into this is warranted. Both fat-soluble (A, D, E and K) and water-soluble (B and C) vitamins can assist athletes in their training programs and support overall health. Specific nutrients of concern in physically active individuals include vitamins C and E, which are known antioxidants and may help to reduce the oxidative damage caused by rigorous training and thereby support a healthy immune system. The wide range of B-vitamins might be ergogenic. A diet containing a variety of foods, and which is sufficient in energy –and macronutrients is likely to be sufficient in meeting the micronutrient requirements of athletes (170).

Minerals are important for all bodily functions. Deficiencies of certain minerals, in particular, have been found to negatively impact on exercise performance (170). These minerals include the following:

- i) Calcium, which reduces the risk of developing premature osteoporosis and maintains body composition;
- ii) Iron, which is essential for transporting oxygen to the tissues from the lungs in the form of haemoglobin and especially for athletes who are prone to iron-deficiency;
- iii) Sodium phosphate, increases maximal oxygen uptake, anaerobic threshold and increase endurance capacity;
- iv) Sodium chloride, which maintains fluid and electrolyte balance; and
- v) Zinc, which decreases exercise-induced changes in immune functioning

There is however little evidence linking improved sport performance to boron, chromium, magnesium or vanadium (170). Athletes at risk of developing micronutrient deficiencies should be identified; this includes athletes deliberately reducing energy intake to lose or maintain body weight; those with high volume intense training programs, and who have low appetite; athletes who are travelling; and those with special circumstances, such as vegetarians and diabetics. In these cases, low risk supplements such as combined multi-vitamin and mineral or liquid meal replacements can be used (170). This should always be done in consultation with a dietitian and an optimal nutrition plan, as well as the athlete's medical doctor.

2.5.1.6 Supplements

Supplements and sports foods are used extensively by athletes at various levels, as well as non-athletes. Although the use of some supplements may have added benefits in terms of improving body composition, sports performance and overall health, the risk/benefit ratio needs to be carefully considered before such supplements are used. There are several excellent reviews on supplements and sport performance, for example Maughan *et al.* (2007) and Burke *et al.* (2009) (185, 186).

2.5.2 Body composition and bone mineral density

Body composition “*attempts to partition and quantify body weight or mass into its basic components*” (187). The determination of body composition can be approached in a variety of ways. Historically, the two compartment model has been the model of choice for dividing body mass into meaningful components (187). This traditional approach has more recently evolved into complex models showcasing at least three or four compartments. The two-compartment model measures fat mass and fat free mass by calculating body density. The three-compartment model allows for the simultaneous measurement of body density and

total body water to derive an estimate of the percentage of body fat. The four component model also includes body density, total body water and bone mineral content to estimate the percentage of body fat. Multicomponent models increase the accuracy of measurements, but due to high costs and technical constraints, the use of these are limited to clinical or laboratory settings (187).

There are numerous ways in which body composition can be estimated, and some of these are complex (188). *In vivo* body composition analysis includes several different methods, such as the following:

- i) Direct or level I methods, for example neutron activation analysis;
- ii) Indirect or level II methods, such as underwater weighing and dual energy X-ray absorptiometry (DXA); and
- iii) Double indirect or level III methods, of which anthropometry and bio-electrical impedance analysis are examples.

DXA, mentioned in (ii) above, is commonly used to determine bone mineral content and bone mineral density. Bone mineral content is used in the four compartment model to increase the accuracy of body composition estimates, and therefore DXA is often used to determine fat free mass, fat mass and the percentage of body fat (187).

DXA is a method for determining body composition using the three-compartment model to estimate bone mineral density, fat mass and fat-free mass (189). It uses dual energies to differentiate the body into three compartments by only taking one measurement. This method is viewed as being reliable and the standard method for determining body composition in athletes, provided that the same DXA instrument is used in all the athletes. It is safe, non-invasive and convenient for those being assessed (189).

Body composition is an important factor in exercise or more specifically triathlon performance. The appraisal of the human physique, better known as Kinanthropometry allows for interpration and monitoring of an athlete's performance (190). In a sport such as triathlon, where body mass must be transported over a distance, a lean physique offers a competitive advantage (190). It is however important to note that ethnicity, heredity and the competitive environment should be taken into consideration in combination with body composition in order to relate it back to sport performance. It is possible for athletes to still be competitive without having the optimal physique; however, documentation of body composition is an important factor when conducting research in the field of exercise physiology / science.

2.6 Conclusion

After careful consideration of the available literature, the researcher is of the opinion that supplementation with caffeine during an endurance event may elicit a different effect from that reported after laboratory-based trials, when subjects are asked to compete in a field trial and specifically during a triathlon. Research results from laboratory studies and single sports, such as swimming, cycling and running cannot be extrapolated to this arena. The effect of caffeine as a CNS stimulant and its effect on fatigue and the stress response, particularly when an athlete is placed in a competitive environment instead of a controlled laboratory experiment need to be taken into consideration. Placing an athlete in a real, competitive environment is likely to attenuate the effect of caffeine supplementation on endurance sport performance, as seen in the literature and summarized in Table 2.1. This is clearly a field that needs to be explored. When taking the pharmacokinetic and pharmacodynamic properties of caffeine into consideration, the potential of caffeine to improve sport performance is evident, but studying it in context is of critical importance.

There is abundant evidence to support the ergogenic effect of caffeine during endurance exercise, but when considering the different conditions in and the physiological requirements of a triathlon, compared to single sporting events, the difference between laboratory –and field-based studies, as well as the other limitations of existing studies that were explored, the researcher identified the need for a double-blind, randomized, crossover, controlled clinical trial in a field setting. This may provide information that can be directly applied to the athletic arena.

The researcher conducted a field study, utilising optimal performance assessment tools, to determine the effect of caffeine supplementation on triathletes and triathlon performance. The study controlled for and incorporated most confounding factors that are important when evaluating the effect of caffeine on sport performance. These include, abstinence from caffeine, withdrawal, caffeine habituation, genetics and menstrual history; as well as factors influencing triathlon performance such as nutritional intake before and during a triathlon, training, medical history, body composition, psychological factors, ratings of perceived exertion and physiological factors, for example the body's response to stress and exhaustive exercise.

Therefore, the main aims of this study were to i) investigate the performance-enhancing or ergogenic effect of caffeine supplementation during a real-life triathlon competition; ii) evaluate several parameters that could in part explain why caffeine supplementation is ergogenic, iii) investigate possible factors influencing the ergogenicity of caffeine

supplementation and iv) investigate possible confounding factors influencing Olympic-distance triathlon performance.

CHAPTER 3: RESEARCH DESIGN AND METHODOLOGY

3.1 Research design

A double-blind, randomized, crossover, controlled clinical field trial was conducted.

A double-blind approach was chosen. The researcher, field workers and subjects were prevented from knowing who was receiving caffeine or placebo during T1 or T2. This approach was chosen to prevent conscious or subconscious bias and thereby invalidating the results. This was especially important due to the “placebo” effect of caffeine (93). The key distinguishing feature of a randomized clinical trial is that the subjects, after being screened according to the inclusion and exclusion criteria, before intervention are randomly allocated to receive one or other of the alternative treatments, this minimizes allocation bias. In this specific trial, the allocation was random and the study had a crossover design, in which subjects received both the treatments. The study was therefore also controlled, as each subject served as his/her own control, thereby increasing the reliability of the results.

3.2 Study population

3.2.1 Sampling

Purposive sampling was used. The researcher targeted a particular group of people as described below. This approach was followed as the desired population for the study was rare and difficult to locate and recruit. All triathletes who were willing to participate in the research project, which gave written informed consent and adhered to the inclusion criteria, were included in the study group. The target population was athletes registered with the governing body for triathlon in the Western Cape region, the Western Province Triathlon Association (WPTA) and athletes who participated at the Western Province triathlon trials and championships during the 2010/2011 triathlon season were invited to participate in the study. A total of 197 senior registered triathletes participated in the 2010/2011 triathlon trials and championships, of which 137 athletes were male and 60 athletes female (<http://www.triathlonsa.co.za>). These figures were used by the biostatistician to estimate sample sizes that would enable sufficient statistical power for the proposed data analysis.

After consultation with the biostatistician it was concluded that a sample size of $N_m = 20$ male and $N_f = 20$ female participants would be representative of the target population. However, to detect statistically significant differences between males and females a larger sample size would have been required. As this was not one of the main aims of the study and because there are known marked differences in the physiology and performance times of male and female triathletes, the researcher did not compare results between these two groups. Even though comparison between males and females was not part of the statistical

analysis, a sample size of $N = 20$ per gender was estimated to be sufficient to yield an effect size of $\delta = 0.5$ between males and females, with 75% power and at a level of significance of 5%, using analysis of variance (ANOVA). The researcher thus set out to attain a sample size of $N = 20$ from each gender.

Research in the field of sport nutrition/medicine is usually done on smaller sample sizes, as it is difficult to find athletes who meet inclusion criteria and because the original pool from which subjects can be recruited is often limited. Therefore, the current study, which sampled 20% of the target population, compares favourably to other sports-related studies in the literature.

The total sample size was $N = 40$ ($N = 20$ from each gender). However, due to withdrawals after triathlon 1 (T1), which was postponed due to poor weather conditions, the final sample size was $N = 26$, of which $N_m = 14$ were male and $N_f = 12$ were female. This was still a significant sample size in terms of power calculation as well as available research published on this topic, with the sample size comprising 13.2% of the target population.

3.2.2 Recruitment of subjects

An extensive marketing campaign was conducted to recruit subjects using an advertisement (Appendix 3.1). This advertisement was distributed at Western Province trials during December 2010, Western Province Championships during January 2011 and 11 Global standard distance triathlons during March 2011. The advertisement was also distributed at the Western Province Triathlon team meeting before the South African Championships in March 2011.

The advertisement was placed on the WPTA website (<http://www.wptriathlon.org.za>) and electronically mailed to all WPTA members *via* the WPTA member database during December 2010 and February 2011. A reminder was sent out *via* this database during April 2011. The advertisement was also sent out to all four registered triathlon clubs in the Western Cape, namely the Atlantic Triathlon Club, Multi-Sport Maniacs, Bike Marathon Triathlon and New Balance Multi-sport Club. The advertisement was also sent out through the Orca database; this is a leading wetsuit and triathlon apparel specialist retailer in the Western Cape.

3.2.3 Inclusion criteria

The following inclusion criteria were developed for this study:

- i) Male and female triathletes, between the ages of 20-60 years, who gave written informed consent, were included.
- ii) Athletes registered with the WPTA or who competed at the 2010/2011 Western Province trials and championships competitions or any triathlete who had completed an Olympic-distance triathlon in the year preceding data collection (2010/2011), were included.
- iii) All the triathletes had to finish both triathlon trials (T1 and T2) within 2% (± 3 minutes) of their usual time. This was to ensure that the athletes were competing at race intensity and that they were completing both triathlons at the same intensity. A previous study found that over a 19-month period, triathletes demonstrated a remarkably stable race performance, with differences of 1.1% being observed in the performance of elite athletes (i.e. the top 10% of the field) and 1.8% for the amateur triathletes (191).
- iv) Abstinence from caffeine or caffeine-containing products for 14 days prior to T1 and T2 was required. Caffeine intake was allowed, however, after the races on the days of T1 and T2, irrespective of whether caffeine supplementation was also used.

3.2.4 Exclusion criteria

The following triathletes were not included in the sample group:

- i) All triathletes not meeting the inclusion criteria;
- ii) All triathletes who participated in a race during the 14 days (two weeks) preceding either T1 or T2; and
- iii) Subjects with known prolactinoma (noncancerous pituitary tumour that produces prolactin) or hyperprolactinemia (elevated serum prolactin).

Menopausal and post-menopausal females were not excluded from the study group. This was, though, documented and statistically analysed according to the known influence of menopause on caffeine metabolism (192). Likewise, females in the late luteal phase of their menstrual cycle were not excluded from the study, but this information was documented and statistically analysed according to the known influence on caffeine metabolism (62). Females who used oral contraceptives were also not excluded from the study. The use of such medication was documented and statistically analysed according to the known influence of these drugs on caffeine metabolism (63, 193).

3.3 Methods of data collection

3.3.1 Data collection

All data was collected by the researcher, Sunita Potgieter, a registered dietitian and lecturer in the Faculty of Medicine and Health Sciences, Stellenbosch University. Research assistants were used on the day of T1 and T2. Research assistants were fifteen BSc. Dietetic I-IV students, three BSc. Physiology students, -and four phlebotomists employed by Pathcare[®], Somerset West who did most of the blood analysis as well.

The research assistants performed different roles. The fifteen dietetic students, for example, were responsible for collecting data from the athletes before, during and after T1 and T2. These students were trained during a three-hour training session on the procedures for data collection on the day of T1 and T2 (Appendix 3.2). Each student received a checklist (Appendix 3.3) to ensure all the required data was collected on both days. It was the responsibility of these research assistants to ensure that the subjects completed all relevant questionnaires, handed in all relevant food and training logs and completed the time points without confusion. Fifteen BSc. Dietetic students were enlisted, to ensure an athlete: research assistant ratio of 2:1 in order for all data to be collected at the specific time points. The research assistants were randomly allocated to the athletes, but the same assistants who collected data from athletes during T1, collected their data again during T2.

The role of the three physiology students, meanwhile, was to measure capillary lactate levels. These students were trained in a private session with the researcher prior to T1 to ensure correct finger prick technique.

The four phlebotomists were responsible for drawing all blood samples before, during and after T1 and T2.

All research assistants were clearly identifiable by wearing the same white T-shirt. The phlebotomists wore their Pathcare[®] uniforms and were thus easily distinguishable.

Figure 3.1 below illustrates the flow of the research study.

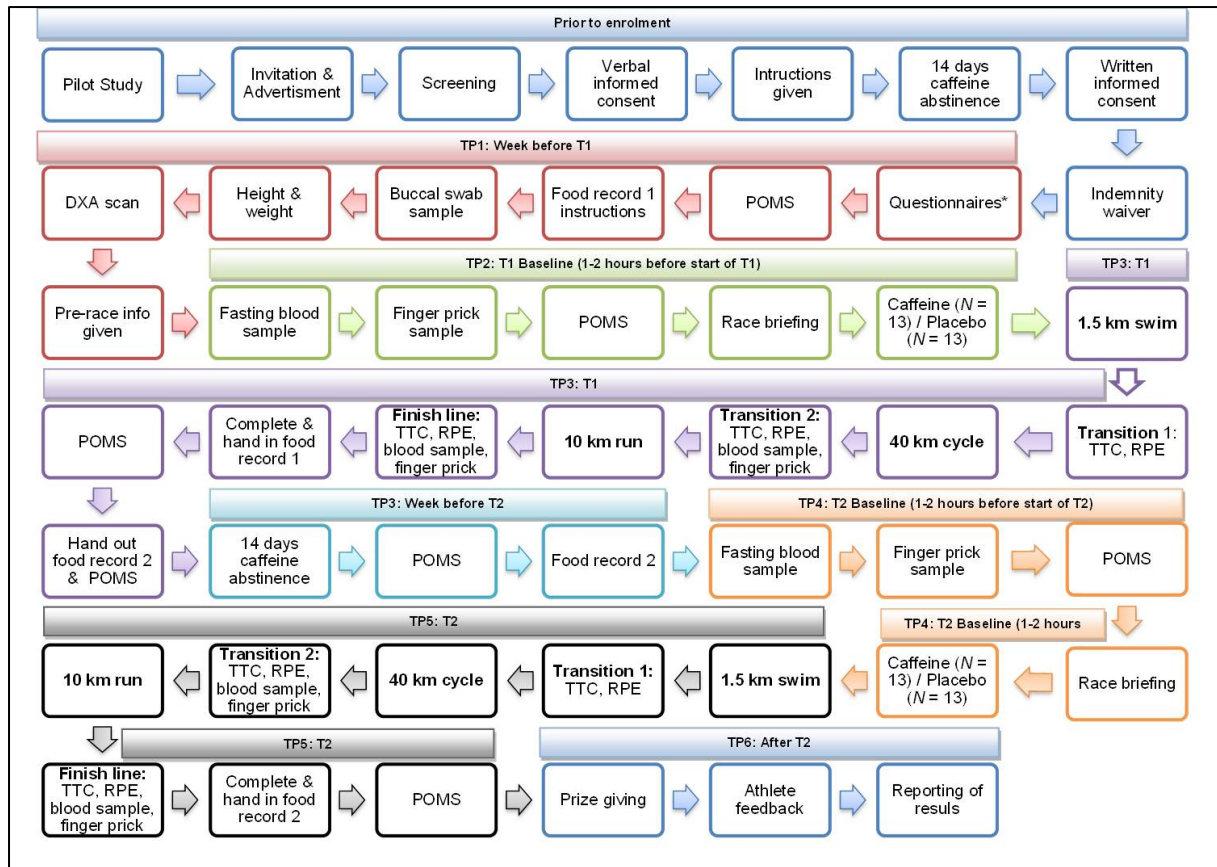


Figure 3.1 Data collection and flow of the research study

TP: Time point; POMS: Profile of mood state questionnaire; DXA: Dual energy X-ray absorptiometry; T1: Triathlon 1; TTC: Time to complete; RPE: Rating of perceived exertion; T2: Triathlon 2

*Questionnaires included demographic, medical, training and menstrual history questionnaires, as well as habitual caffeine intake questionnaire

3.3.2 Pilot study

A pilot study was necessary to enable the researcher to manage and optimise the logistics regarding the organization and flow of the research study and to ensure that blood samples could be obtained from athletes during a triathlon. It was also important to establish how long it would take the phlebotomists to draw blood samples, because a blood sample needed to be taken during transition 2 (cycle → run). If the phlebotomists took too long (> 2-3 minutes) with the venipuncture/phlebotomy procedure, the amount of time the athlete had to rest would be increased and this could have possibly affected the outcome of the study.

The pilot study was conducted on 4 December 2010. The participants included runners, cyclists and swimmers who were excluded from the study population. Six athletes (male $N_m = 3$, female $N_f = 3$) were recruited to participate in the pilot study (i.e. 23% of the total study population).

The pilot study consisted of a sprint distance triathlon. This was decided upon, as the actual distance raced was not important during the pilot study. The aim of the pilot study was to assess and implement all the logistic arrangements in order to collect all the necessary data. The pilot study consisted of a 750 m swim, 20 km cycle and 5 km run, held at a gym in the Helderberg area (Virgin Active gym[®], Somerset West). The researcher obtained written informed consent from all six athletes before participating in the triathlon. Pilot study participants were given information on the RPE scale, a week before the pilot study so that they could be familiar with this. The weight of each athlete was measured and all four questionnaires (i.e. medical, demographic, habitual caffeine intake and exercise regime questionnaires) were piloted to ensure face and content validity. The research study checklist (Appendix 3.3) was also used during the pilot study to ensure the efficient flow of time points during the research study.

The subjects were asked to complete a questionnaire regarding the simplicity of the questionnaires and whether these were easy to understand. Minor changes with regard to spelling and grammar were made to the questionnaires, following feedback from the subjects.

The research assistants were asked to complete a questionnaire regarding the simplicity of the checklist and whether it was adequate to collect all the data. The research assistants all concluded that the checklist was sufficient to collect the necessary data and easy to use.

The phlebotomists were asked to take blood samples before, during the transition from the cycle to the run and at the finish line of the sprint triathlon. They were also asked to complete a questionnaire on the ease of drawing the blood samples and the time taken to take the blood samples were timed by the research assistants. The phlebotomists from Pathcare[®] all indicated that it was easy to draw blood samples from all the subjects and that it took an average of 40 seconds to locate the vein and take the blood samples. The phlebotomists all concluded that this was an easy, safe and accurate way to draw blood samples from the subjects during a triathlon.

3.3.3 Setting

Data was collected during two triathlons which were held 14 days apart (22 May and 5 June 2011) at Gordon's Bay beach, Western Cape Province, South Africa. Both triathlons consisted of a sequential 1.5 km swim, 40 km cycle and 10 km run. The triathlons were

originally scheduled for 8 and 22 May 2011, but due to poor weather conditions (gale force wind; south-westerly at 60 km/hour), the first event was postponed.

The WPTA was contacted to assist with the organization of the triathlons and logistical arrangements. Both triathlons were sanctioned by WPTA and adhered to the standard Triathlon South Africa (TSA) and International Triathlon Union (ITU) guidelines for a triathlon (8). This included all aspects regarding safety, rules and regulations. A race briefing session was held the morning before T1 and T2, in which the researcher explained the route of the triathlon to the participants. All ITU rules and regulations were discussed with the athletes by the triathlon referee from WPTA. The triathlons were non-draft legal races and the subjects were asked to race as hard and fast as they could.

3.3.4 Weather

Weather conditions on the days of both triathlons (22 May and 5 June 2011), were relatively similar, with little or no difference between the two days, as shown in Table 3.1.

Table 3.1 Summary of actual weather conditions during T1 and T2

Weather condition	T1: 22 May 2011	T2: 5 June 2011
Lowest temperature (°C)	12°C	10°C
Highest temperature (°C)	17°C	15°C
Wind speed	11-20 km/h	6-20 km/h
Wind direction	Gentle north-west (NW)	North
Humidity	35%	65%
Dew point	4	10
Comfort level	20°C	15°C
Chance of rain	63%	35%
Actual rain	0% 0.0mm	30% 4.0mm
Sunrise	7:34AM	7:43AM
Sunset	5:47PM	5:42PM
Moonrise	10:49AM	10:33AM
Moonset	11:51PM	9:18PM
Moon phase	Waning Gibbous	Waxing Crescent
Comments	Cloudy	Cloudy with light rain throughout the day
Tide*	Low tide: 00:27AM-6:32AM High tide: 6:32AM-12:38PM Low tide: 12:38PM-19:03PM	Low tide: 05:16AM-11:23AM High tide: 11:23AM-17:42PM Low tide: 17:42PM-23:52PM

Source: Weather 24 (<http://www.weather24.com>) and Weather SA (<http://www.weathersa.co.za>), South African Tide Tables (SA Navy) 2011 (<http://www.sanho.co.za>)

*Tide influenced the swim start in ocean. Swim distance (1.5 km) was measured via GPS by the National Sea Rescue Institute (NSRI) Gordon's Bay. Swim distance was equal for both T1 and T2. During low tide, the swim start (first buoy) was deeper in the sea than during high tide in T1.

3.3.5 Event plan

An event plan (Appendix 3.4) was submitted to the City of Cape Town, Economic and Human Development; Film and Events Permit Office on 15 March 2011. Final approval to host the triathlons in Gordon's bay was given by the City of Cape Town on 6 May 2011 (Appendix 3.5).

3.3.6 Caffeine habituation, washout and withdrawal

All triathletes were instructed to abstain from using any caffeine or caffeine-containing products, including food and drinks, for 14 days prior to T1 and T2. This time period was regarded as being sufficient in allowing any withdrawal symptoms to pass.

Although some studies (194) have reported enhanced exercise performance regardless of caffeine withdrawal, it has also been documented that with caffeine habituation, there is an increased amount of caffeine needed to elicit the same effect on the CNS. It is therefore important to control for subject habituation (195). Previous studies on caffeine supplementation involved subjects abstaining from caffeine for periods of between 2-4 days (Table 2.1). In the current study, subjects were asked to abstain from caffeine and caffeine-containing products for 14 days prior to T1 and T2, in order to optimize the effect of caffeine and reduce the chance of any carry-over effect.

Caffeine withdrawal during the period of caffeine abstinence was expected in subjects who habitually use > 300 mg caffeine per day. Symptoms of caffeine withdrawal include headaches, fatigue, lethargy and flu-like symptoms (26, 41, 100). Caffeine withdrawal symptoms were recorded during the study trial and are reported in Chapter 4.

3.3.7 Recovery between T1 and T2

There was a 14-day period between T1 and T2 to ensure athletes have fully recovered and were able to race at the same intensity for both trials. The athletes were not allowed to compete in another race for 14 days prior to T1 and they were instructed to schedule both events as races/competition in their training programmes. This was important in order for athletes to be fully recovered for the triathlons and to prevent race-fatigue.

The athletes also completed the Profile of Mood States questionnaire in the week before T1 and T2, as well as on the morning of and after T1 and T2. Any significant changes in mood state were documented and incorporated into the statistical analysis.

3.3.8 Blinding and randomization

Each subject was given a subject number for the duration of the study. A computer was used to randomly select the subject numbers of the subjects to be in the caffeine and placebo groups during T1. Thirteen subjects was assigned to the caffeine and the placebo groups respectively during T1, while for T2 the groups crossed over and those receiving caffeine during T1 now received placebo and *vice versa*.

Since this was a double blinded study, an independent laboratory (African Micronutrient Research Group, ward A10, Tygerberg Hospital) was contracted to randomize the groups, as well as to prepare the caffeine and placebo capsules. This ensured that both the researcher and the subjects were blinded as to who received caffeine or placebo. After T2, the list was made available to the researcher, who relayed the results to the subjects.

3.3.9 Intervention

Microencapsulated caffeine (70% caffeine concentration, certificate of purity, Appendix 3.6) was used for caffeine supplementation. *“Microencapsulation is a process in which tiny pieces of an ingredient are packaged, or encapsulated, within another material in order to protect the active ingredient from the surrounding environment. Capsules can range in size from one-thousandth of a millimetre to seven millimetres”* (Maxx Performance Inc.® 2005-2009).

In a recent systematic review by Ganio *et al.* (2009), the author concluded that caffeine supplementation was “*equally ergogenic*” and independent of the delivery mode of caffeine (41). Microencapsulation is effective for masking the taste of caffeine (pure caffeine has a bitter taste) and does not influence its bioavailability or the time taken to reach peak plasma concentrations. The microencapsulated caffeine and the placebo were placed in gelatin capsules, which are easily broken down in the stomach by the presence of hydrochloric acid, minimizing the effect of the capsule on the absorption of caffeine.

The caffeine-containing capsules included an individually weighed dose of 6 mg/kg body weight caffeine, which was consumed as 8.6 mg/kg body weight microencapsulated caffeine to account for the fact that the microencapsulated caffeine contained 70% caffeine. The placebo contained an artificial sweetener (Canderel®) that resembled a white powder and did not contribute any caloric value. Due to the microencapsulation, the caffeine powder had no taste. The artificial sweetener tasted sweet. Both the caffeine and the artificial sweetener were placed in gelatin capsules so no taste could be observed. Both looked like a white

powder. The athletes were instructed not to open the capsules before consuming it and the research assistants monitored the athletes taking the supplementation as to ensure no one opened the capsules and they take it at the correct time before the race. The placebo was not able to influence triathlon performance.

Using capsules of the same colour, shape and size, containing the white powder (either caffeine or artificial sweetener) ensured that neither the athletes nor the researcher were able to distinguish between the caffeine and placebo-containing formulations. The optimal concentration of plasma caffeine is seen 45 minutes to 1 hour after ingestion and therefore, both the caffeine- and placebo-containing capsules were administered to the athletes one hour prior to the start of T1 and T2.

3.4 Research instruments and data analysis

3.4.1 Ergogenic effect of caffeine supplementation

Plasma caffeine levels were analyzed in order to determine if subjects adhered to the caffeine-abstinence protocol, as well as if peak plasma caffeine levels were reached during T1 and T2. Plasma caffeine levels were measured at baseline, transition (cycle → run) and at the finish line. Details of blood collection are summarized in section 3.4.2.1.

3.4.1.1 Triathlon performance

The time it took the subjects to complete the various components of the triathlon, as well as the overall time to complete T1 and T2 were recorded. The WPTA was contracted to record all the time-splits during T1 and T2, according to standard TSA and ITU guidelines. The subjects were each given a race number (identical to the subject number), which the subject wore around his/her waist during the triathlon with a race belt, which was provided by the researcher. Time splits were recorded for the swim + transition 1, cycle, run + transition 2 and overall time to complete T1 and T2. The research assistants showed the subjects where to go to have the blood samples collected during transition 2 (cycle → run) and also recorded the time taken to draw the blood sample on the checklist provided. This time was subtracted from the run + transition 2 time (and the overall time), to give a time without blood sample collection. This was especially important when determining who had won the prize money.

3.4.1.2 Rating of perceived exertion

Caffeine has been shown in various research studies to influence a subject's rating of perceived exertion. This research study used the Borg Scale Rating of Perceived Exertion (RPE) to determine the RPE at various time points during T1 and T2. The scale rates how strenuous or heavy the exercise feels and depends mainly on the strain and fatigue felt in the muscles and the subject's feeling of breathlessness or aches in the chest. It is a 15-point scale, in which "6" corresponds to very light exercise, such as walking slowly, and "20" corresponds to maximal exertion (196).

During the initial visit, the week before T1, the researcher explained the Borg RPE scale to the subjects, who were each given a copy of the Borg scale RPE (Appendix 3.7) with which to familiarize themselves prior to T1 and T2. During T1 and T2, the research assistants prompted the subjects to rate their perceived exertion. The subjects were asked to rate their feelings as honestly as possible, without thinking about what the actual physical load is. These RPE values were given during transition 1 (swim → cycle), when the subjects were in the transition area, preparing for the cycle leg, during transition 2 (cycle → run), when the subjects were in the transition area, preparing for the run leg and directly after the race at the finish line, and were recorded on the checklist by the research assistants.

Rating of perceived exertion was analysed as the scores given by subjects at the various time points before and during the triathlons.

3.4.1.3 Mood state

The profile of mood states (POMS) scale is a 65-item, self-assessment questionnaire using a Likert scale. In this study, the revised, shorter version of the POMS questionnaire was used; this is similar to the above questionnaire, but only 24 items are included. The POMS scales have been extensively used in sport psychology research (197). The original questionnaire was developed by McNair and Lorr in *et al.* (1971) (198) and revised in 1992 (199). Questions are rated on a scale of 0 (not at all) to 4 (extremely) and measure mood disturbances and mood states, namely tension-anxiety, depression-dejection, anger-hostility, vigour-activity, fatigue-inertia and confusion-bewilderment. The shortened version of the POMS used in this study only assessed tension, vigour and fatigue.

The subjects were asked to complete the POMS questionnaire (Appendix 3.8) at baseline and again immediately after T1 and T2 in order to determine the effect of caffeine supplementation on mood state.

All the emotions listed on the POMS questionnaire were scored. The original 65 item questionnaire was adapted to a shortened 24-item POMS questionnaire. The scoring system was thus adapted for this shortened questionnaire, as described below.

Subscales were calculated for the parameters of tension, fatigue and vigour in the following manner:

- i) The tension subscale was scored by adding the awarded score that was circled for the following emotions: tense, shaky, on edge and panicky. The scores for relaxed, uneasy, restless, nervous and anxious were reversed (subtracted).
- ii) The fatigue subscale was scored by adding the awarded score that was circled for the following emotions; worn out, listless, fatigued, exhausted, sluggish, weary and bushed.
- iii) The vigour subscale was scored by adding the awarded score that was circled for the following emotions: lively, active, energetic, cheerful, alert, full of pep, carefree and vigorous.

The total mood disturbance (TMD) was scored by adding all the raw scores from tension and fatigue, and subtracting the score from vigour. The TMD score was interpreted as the lower the score; the more positive the mood.

3.4.2 Parameters that could in part explain the ergogenicity of caffeine supplementation

3.4.2.1 Endocrine-stress response, oxidative stress and plasma lactate

Collection of blood samples

Subjects were instructed to arrive at T1 and T2 in the fasted state, as baseline blood samples needed to be taken in this state. The subjects were able to bring their pre-event meal with and were provided sufficient time before the start of T1 and T2 to eat this meal (baseline blood samples were collected 90 minutes before the start of T1 and T2). All blood samples were collected by registered phlebotomists from Pathcare®, Vergelegen Medi-Clinic, Somerset West, South Africa. Four phlebotomists were available to collect blood samples during T1 and T2.

Blood samples were collected according to the standard operating procedures of Pathcare®, South Africa. Table 3.2 summarizes when the blood samples were taken and the parameters

measured. The maximum amount of blood drawn during all time points (baseline, transition (cycle → run) and finish line) per triathlon was 90 ml.

Table 3.2 Summary of blood parameters measured

Parameter measured:	Time points at which blood samples were taken (T1 and T2)	Vacutainer used:
Plasma caffeine (cold)	Baseline, Transition 2, Finish line	EDTA purple top
Full blood count ^a	Baseline, Finish line	EDTA purple top
Plasma lactate ^b	Baseline, Transition 2, Finish line (immediately, 3, 6, 9, 12 and 15 minutes post-T1 and post-T2)	Finger prick capillary sample Accutrend [®] Plus, Roche
Serum cortisol	Baseline, Finish line	SST tube
Serum albumin ^c	Baseline, Transition 2, Finish line	SST tube
Total testosterone	Baseline, Finish line	SST tube
DHEAs	Baseline, Finish line	SST tube
Serum prolactin	Baseline, Finish line	SST tube

^aA full blood count (FBC) sample was taken and analysed to determine the total and differential leukocyte count, the haematocrit and haemoglobin. Leukocytes are the main cells of the immune system. Leukocytes can be divided into six groups, namely: eosinophils, basophils (and mast cells), neutrophils, monocytes (and macrophages), lymphocytes (plasma cells and cytotoxic T cells) and dendritic cells (not found in the blood).

^bAccu-check[®] Safe-T-Pro Uno lancet (Roche) and Accutrend[®] Lactate (BM-Lactate, Roche[®]) test strips were used to test lactate levels.

^cSerum albumin is produced by the liver and exerts an osmotic effect to maintain water balance between blood and tissue. Serum albumin levels in the present study were determined to detect any changes in plasma volume that might occur due to exercise and to adjust other biochemical parameters accordingly.

All blood samples, except for the full blood count sample, were collected and batched by a commercial pathology laboratory (Pathcare[®]) for analysis using standardised, accredited techniques and procedures. The full blood count sample, was collected and batched by Pathcare[®], but sent to the Department of Physiological Sciences, Faculty of Science, Stellenbosch University for analysis.

Plasma caffeine was analysed by high performance liquid chromatography (HPLC), using a Waters[®] instrument (Waters Corp[®], Milford, Massachusetts). Serum Albumin was analysed by Roche[®] Bromocresol Green (BCG) with a Roche[®] Modular instrument (Roche Diagnostics[®], Indianapolis, IN). Serum cortisol and prolactin were analysed by chemiluminescence with a Beckman[®] DXI 800 instrument (Beckman Coulter Inc., Fullerton, California) while total testosterone and DHEAs levels in serum were analysed by electrochemiluminescence with Roche[®] Elecsys 2010 (Roche Diagnostics[®], Indianapolis, IN).

The Accutrend[®] Plus instrument (Accu-Chek[®], Roche Diagnostics[®], Indianapolis, IN) was used for the quantitative assessment of blood lactate concentrations. The reflectance photometric measurement was performed using test strips for blood lactate.

The full blood count analysis was performed by a fully automated procedure (Celldyne 3700CS, automated hemocytometer, Abbott Diagnostics®, Germany). Daily control repeatability tests were completed before each analysis. An experienced haematologist reviewed the data for validity

The results of all the above mentioned blood analysis were interpreted using the standard reference values as shown in Table 3.3.

Table 3.3 Reference values for different components of blood

Parameter	Reference value (Male)	Reference value (Female)
Plasma caffeine	5 – 25 mg/l	5 – 25 mg/l
Serum albumin	35 – 50 g/l	35 – 50 g/l
Serum cortisol	140 – 700nmol/l	140 – 700 nmol/l
Serum prolactin	2.6 – 13.1 ug/l	3.3 – 26.7 ug/l
Total testosterone	9.9 – 27.8 nm/l	0.22 – 2.0 nm/l
DHEAs	0.95 – 11.7 umol/l	2.17 – 15.2 umol/l
White blood cell count	4 – 11 X 10 ⁹ /l	4 – 11 X 10 ⁹ /l
Relative neutrophil count (%)	40 – 75 %	40 – 75 %
Absolute neutrophil count	2 – 8 X 10 ⁹ /l	2 – 8 X 10 ⁹ /l
Relative lymphocyte count (%)	20 – 45 %	20 – 45 %
Absolute lymphocyte count	1.0 – 4.0 X 10 ⁹ /l	1.0 – 4.0 X 10 ⁹ /l
Relative monocyte count (%)	2 – 10 %	2 – 10 %
Absolute monocyte count	0.0 – 1.0 X 10 ⁹ /l	0.0 – 1.0 X 10 ⁹ /l
Relative eosinophil count (%)	1 – 6 %	1 – 6 %
Absolute eosinophil count	0.0 – 0.5 X 10 ⁹ /l	0.0 – 0.5 X 10 ⁹ /l
Relative basophil count (%)	0 – 1 %	0 – 1 %
Absolute basophil count	0.0 – 0.2 X 10 ⁹ /l	0.0 – 0.2 X 10 ⁹ /l
Red blood cell count	4.5 – 6.6 X 10 ¹² /l	3.8 – 5.8 X 10 ¹² /l
Hemoglobin	13.0 – 18.0 mg %	11.5 – 16.5 mg %
Haematocrit	40.0 – 54.0 %	38.0 – 47.0 %
Platelets	150 – 400 X 10 ⁹ /l	150 – 400 X 10 ⁹ /l
Plasma lactate	1.00 – 1.78 mmol/l	1.00 – 1.78 mmol/l

Source: Ranges of normality. Department of Chemical Pathology, Tygerberg Hospital, Tygerberg. South Africa, 2000 and Pathcare®

All reference values refer to a sedentary person, 18 years or older

3.4.3 Factors influencing the ergogenic effect of caffeine supplementation

3.4.3.1 Caffeine habituation

Habitual caffeine intake was determined by the subjects completing a habitual caffeine intake food frequency questionnaire (Appendix 3.9) *via* an Internet-based electronic survey (Survey Monkey®). The questionnaire was tested for face and content validity during the pilot study. The subjects completed this questionnaire during the initial research visit before T1.

Habitual caffeine intake was described as low if it was ≤ 50 mg/day and as high if it was ≥ 300 mg/day (72).

The subjects were instructed to refrain from all caffeine and caffeine containing products for two weeks prior to T1 and T2. The subjects were given a copy of these foodstuffs and it was verbally explained to them (Table 3.4). The subjects were allowed to ingest caffeine-containing products after the races on the days of T1 and T2, irrespective of whether they had received caffeine or the placebo during the trial. This was only allowed as a reward, on the day of T1 and T2 as neither the subjects, nor the researcher knew who received caffeine. The subjects were asked to follow the same dietary patterns before T1 and T2.

Table 3.4 Caffeine containing foodstuffs that the athletes were instructed to avoid for 14 days prior to T1 and T2

- | |
|--|
| <ul style="list-style-type: none"> • Any form of coffee (instant coffee, filter coffee, percolated etc.). Decaffeinated coffee was also not permitted, as this contains small amounts of caffeine. • Ceylon tea, black tea and green tea. Rooibos tea was permitted. • All soft drinks such as Coke[®], iced tea etc. Only Tab[®] was allowed as it is caffeine free. All other soft drinks containing caffeine were excluded. • Chocolate bars as well as ice-cream containing coffee / chocolate. • Cocoa and drinks such as hot chocolate, Milo[®] and Horlicks[®]. • Energy gels and energy drinks containing caffeine (such as Gu[®]). If the subject was unsure of the caffeine content of a product, the subject was requested to ask the researcher to check this to ensure that it did not contain any caffeine. • Vitamin water[®] (energy flavour), which also contains caffeine, and was avoided. • Energy drinks such as Red Bull[®]. • Breathe fresheners and chewing gum containing caffeine. • Weight loss pills as well as pain relievers such as Grandpa[®]. |
|--|

3.4.3.2 Pre-event meal

The pre-event meal may have influenced the ergogenic effect of caffeine supplementation. Therefore, it was important to note what the athletes ingested as their pre-event meal. The subjects arrived at the races in the fasted state in order to provide fasted baseline blood samples. Thereafter they had sufficient time to eat their pre-event meal. Details of the pre-event meal were recorded as part of the food record described in Section 3.4.4.2.

3.4.2.3 Menstrual cycle, oral contraceptive use and menopause

The menstrual history questionnaire (Appendix 3.10) formed part of the demographic information questionnaire. Details of this questionnaire are described in Section 3.4.4.1.

3.4.2.4 Genetic analysis

Collection and extraction of genomic DNA for genetic analysis

Because of the non-invasiveness and ease of obtaining and storing buccal samples, these were preferred for genomic DNA extraction in this study. Buccal swabs were collected from

each patient by the researcher, during the week before T1, using regular flocked 80 mm buccal swabs (Lasec SA[®] www.lasecsa.co.za) as described by the manufacturers (Table 3.5). These collection instructions were explained to the subjects whilst obtaining written informed consent.

The samples were transported immediately to the Department of Genetics, Faculty of AgriSciences, Stellenbosch University and were stored at room temperature for 1-2 weeks prior to DNA extraction. Thereafter, the samples were stored at -20°C for re-extractions. The laboratory at the Department of Genetics extracted the DNA and analysed the samples according to the standard techniques and procedures outlined in the Invitrogen Purelink[®] Genomic DNA kit.

Table 3.5 Collection instructions for buccal swab samples

<p>Step 1: Subjects abstained from eating or drinking for approximately 30 minutes prior to providing the sample</p> <p>Step 2: Alternatively, gently brush the inside surface of both cheeks with a toothbrush (without toothpaste) (Step 1 above is preferred over step 2)</p> <p>Step 3: Thoroughly rinse the mouth twice with water</p> <p>Step 4: Roll the swab firmly on the inside of the cheek approximately 20 times on each side</p> <p>Step 5: Make sure to brush over the entire cheek</p> <p>Step 6: Allow the swab to dry at room temperature ($\pm 25^{\circ}\text{C}$) for 10-15 minutes, taking care not to touch the tip.</p> <p>Step 7: Place the swab in the original packaging for transportation</p> <p>Step 8: The swab is stable for up to one week stored at a temperature of between 22°C and 37°C</p> <p>Source: www.genediagnosics.co.za</p>

DNA extraction and purification were subsequently completed as recommended in the Laboratory Protocol for Manual Purification of DNA (Invitrogen PureLink[®] Genomic DNA kit) (Table 3.6).

Table 3.6 DNA extraction / purification**Step 1: Preparing human buccal swab lysate:**

- Prepare lysate from human buccal cell swabs as described below
- Set a water bath or heat block at 55°C
- Place the buccal swab in a sterile, 2 ml microcentrifuge tube
- Add 400 µl (for cotton and Dacron swab) or 600 µl (for Omni swab) phosphate buffered saline (PBS) to the sample
- Add 20 µl Proteinase K into a sterile micro centrifuge tube capable of holding three times the volume of lysate
- Transfer 200-600 µl swab lysate to the micro centrifuge tube containing Proteinase K (Step 3). Mix well by pipetting
- Add an equal volume of Purelink® Genomic Lysis/Binding Buffer to the lysate and mix well by brief vortexing.
- Incubate at 55°C for at least 10 minutes
- Centrifuge briefly to collect any lysate from the tube caps
- Add 200 µl 96-100% ethanol to the tube
- Mix well by vortexing for 5 seconds to yield a homogenous solution

Step 2: Binding DNA

- Remove a Purelink® spin Column in a Collection Tube from the package
- Add the lysate (~640 µl) prepared with Purelink® Genomic Lysis/Binding Buffer and ethanol to the Purelink® Spin Column
- Centrifuge the column at 10,000 x g for 1 minute at room temperature
- Discard the collection tube and place the spin column into a clean Purelink® Collection Tube supplied with the kit

Step 3: Washing DNA

- Add 500 µl Wash Buffer 1 prepared with ethanol to the column
- Centrifuge column at room temperature at 10,000 x g for 1 minute
- Discard the collection tube and place the spin column into a clean Purelink® collection tube supplied with the kit
- Add 500 µl Wash Buffer 2 prepared with ethanol to the column
- Centrifuge the column at maximum speed for 3 minutes at room temperature
- Discard the collection tube

Step 4: Eluting DNA

- Place the spin column in a sterile 1.5-ml micro centrifuge tube
- Add 25-200 µl of Purelink® Genomic Elution Buffer to the column.
- Parameters to choose the suitable elution volume for your needs
- Incubate at room temperature for one minute. Centrifuge the column at maximum speed for one minute at room temperature. The tube contains purified genomic DNA
- To recover more DNA, perform a second elution step using the same elution buffer volume as first elution in another sterile 1.5 ml micro centrifuge tube
- Centrifuge the column at maximum speed for 1-0.5 minutes at room temperature
- The tube contains purified DNA
- Remove and discard the column

Step 5: Storing DNA

- Store the purified DNA at -20°C or use DNA for the desired downstream application.

Source: Invitrogen PureLink® Genomic DNA

Quality and quantity assessment of isolated genomic DNA

After isolating the genomic DNA (gDNA) of each athlete from buccal swabs using the Invitrogen PureLink® Genomic DNA extraction kit, DNA concentrations were assessed by spectrophotometric analysis at an absorbance of 260 nm using Nanodrop technology (NanoDrop® ND-100, Nanodrop Technologies Inc., Wilmington, Delaware, USA). DNA integrity and quality was confirmed by loading approximately 100 ng of DNA on 0.6%

agarose gels and subsequent electrophoresis at 80 V for 30 minutes. Hyperladder V (Bioline, London, UK) was used as a molecular weight marker to facilitate estimation of gDNA size. Concentrations for the samples used in this study ranged between 20 ng/μl and 60 ng/μl, and total yield from 500 to 1500 ng. DNA quality was satisfactory for all samples. Dilutions for all samples were prepared to yield a concentration of 25 ng/μl for use in all further analysis.

Genomic DNA from all patients was analysed using the polymerase chain reaction (PCR) followed by restriction enzyme analysis to identify the presence of ancestral and/or variant *CYP1A2* alleles.

A total of five different polymorphisms were studied. PCR primers for *CYP1A2*1F* (rs762551) and *CYP1A2*1D* (rs35694136) were obtained from Tiwari *et al.* (2005) (200) and Sachse *et al.* (2003) (201), while primers for the SNPs between *CYP1A1* and *CYP1A2* (rs2472297 and rs2470893) and the SNP near the *AHR* gene (rs6968865), discussed in Cornelis *et al.* (2011) (164) and Sulem *et al.* (2011) (163), were designed using PrimerQuest, incorporating Primer3 as described by Rozen *et al.* (2000) (202) (<http://eu.idtdna.com/Scitools/Applications/Primerquest/>). BLASTn was used to confirm primer specificity and homology (<http://blast.ncbi.nlm.nih.gov/>). Primer reaction mixtures, sequences and PCR product sizes are shown in Table 3.7 and 3.8. PCR amplifications were performed using a Geneamp PCR System 2700 (Applied Biosystems, Warrington, WA, USA). The PCR amplification conditions were as follows: initial denaturation at 94°C for 5 min, followed by 30 cycles of 94°C for 30 s, annealing temperature for 30 s, 72°C for 30 s.

Table 3.7 PCR reaction mixtures for all amplicons

	Stock concentration	Final concentration	Volume per reaction (μl)
DNA	15-30 ng/μl	15-30 ng/μl	1
Buffer	10X	1X	2.5
MgCl ₂	50 mM	2 mM	1
dNTPs	10 mM	0.32 mM	0.8
Forward Primer	10 pmol/μl	0.4 mM	1
Reverse Primer	10 pmol/μl	0.4 mM	1
BIOTAQ [™] polymerase*	5 U/μl	0.5 U	0.1
dH ₂ O			17.6
Final Volume			25

* Bioline, Randolph, MA, USA

Table 3.8 PCR primer sequences used to amplify gene variants

An underlined nucleotide indicates a base change to incorporate a restriction enzyme site

Variant location	rs number	DNA sequence [Ancestral/ Variant base]	Primer name	Primer sequence (5'-3')	T _A	PCR Product size (bp)
-163C>A (<i>CYP1A2*1F</i>)	rs762551	tgggc[A/C]cagga	<i>CYP1A2*1FF</i>	TGGAGTGGTCAC TTGCCTCT	58°C	520
			<i>CYP1A2*1FR</i>	CTGGCTCATCCTT GACAGT		
-2467T/- (<i>CYP1A2*1D</i>)	rs35694136	gcaca[T/-]gaaccc	rs35694136 dTF	TGAGCCATGATTG TGGCATA	54°C	167
			rs35694136 dTR	AGGAGTCTTTAAT ATGGACCCAG		
Between <i>CYP1A1</i> & <i>CYP1A2</i>	rs2472297	taatg[C/T]ctctt	rs2472297 TF	AGATGGAGGGCA GTGGAGATGAAA	60°C	384
			rs2472297 TR	ACATTCTAACCAG GGCGGAACACT		
Between <i>CYP1A1</i> & <i>CYP1A2</i>	rs2470893	ccagc[G/A]cctcc	rs2470893 F	TGTATTTGCGTGC CTAGCTCAACC	60°C	447
			rs2470893 R	ATTCTTGACTCCA CACTCCTGCCT		
Near <i>AHR</i> gene	rs6968865	ggaga[A/T]atctc	rs6968865 F	ACCGGAAGCTGG TAGATCAGAAGT	60°C	228
			rs6968865 R	ACACCACAGCAAT CAACACAGCA		

Restriction enzyme analysis:

All SNPs were genotyped by PCR – restriction fragment length polymorphism (PCR-RFLP) analysis. The restriction enzyme digestion was performed in a total volume of 20 µl containing PCR product (10 µl), appropriate buffer and at least 2 units of the specific restriction enzyme (New England Biolabs Inc., Ipswich, Massachusetts, USA) (Table 3.6). Samples were incubated overnight at the optimum temperature of the enzyme and heat inactivated according to the enzyme specifications (Table 3.9).

Restriction enzyme digested PCR products were analysed on a 2.5% (w/v) agarose gel containing ethidium bromide at 80 V for 3 hours. 1X TBE (0.089 M Tris, 0.089 M Boric acid and 20 mM EDTA, pH 8.0) was used as electrophoresis buffer and DNA fragments were visualised with UV light. The fragment sizes of the respective alleles detected are indicated in Table 3.9.

Table 3.9 Restriction enzyme assays for the genotype analysis

rs number	Recognition site	Restriction enzyme	Enzyme conditions	Genotype	Fragment sizes (bp)
rs762551	GGGCC*C	ApaI	25°C for 16 hr	A/C	A: 520
			65° for 20 min		C: 373 and 147
rs35694136	CA*TATG	NdeI	37°C for 16 hr	T/-	T: 148 and 19
			65° for 20 min		-: 167
rs2472297	GTCTC(N) ₁ *	BsmAI	55°C for 16 hr	C/T	C: 384
			80° for 20 min		T: 261 and 123
rs2470893	RGCGC*Y R=A or G, Y=C or T	HaeII	37°C for 16 hr	G/A	A: 447
			80° for 20 min		G: 240 and 207
rs6968865	GAT*ATC	EcoRV	37°C for 16 hr	A/T	A: 228
			80° for 20 min		T: 178 and 50

3.4.4 Factors influencing triathlon performance

3.4.4.1 General health (FBC, questionnaire, mood state)

Components of the FBC detailed in section 3.4.2.1 were used to interpret the general health of the athletes.

In the week before T1, the subjects were sent an e-mail by the researcher, in which an Internet link to the baseline questionnaires (Survey Monkey[®]) was provided. This was more convenient for subjects and simplified data collection and data capturing before both triathlons. The questionnaires were all completed in the week before T1 and data was directly exported to Microsoft Office Excel (2010) for Windows 7[®], saving time and money. The questionnaires were tested for face and content validity during the pilot study. All the questionnaires were available in English and Afrikaans as this was the primary languages of the target population. Completing all the questionnaires did not take more than 30 minutes and all subjects had internet access.

The following questionnaires were completed by means of this online survey:

- i) Demographic information (Appendix 3.11);
- ii) Training regime (Appendix 3.12);
- iii) Medical history (Appendix 3.13); and
- iv) Menstrual history (Appendix 3.10)

The subjects were also asked to complete the POMS questionnaire (Appendix 3.8) in the week before T1 and T2. The questionnaire was used as a tool to establish whether or not

the athletes had recovered adequately between T1 and T2, as well as to determine the effect of mood state on overall triathlon performance.

3.4.4.2 Energy- and nutrient intake two days before as well as dietary strategies followed on race day

Dietary intake was determined two days before each triathlon, the morning of each triathlon (i.e. the pre-event meal) and during T1 and T2. The subjects were instructed to follow their normal dietary patterns before a race, with the exception of excluding all the caffeine-containing products for 14 days prior to T1 and T2.

To determine dietary intake two days before, as well as on the morning of and during T1 and T2, the subjects completed two three-day food records using a standardized food record form (Appendix 3.14). The three-day food records were completed during the two days before T1 and T2, as well as on race day. The subjects were instructed to complete the food records on race day (day three) for their pre-event meal/breakfast as well as what they ingested during the race. The research assistants checked each food record for completion.

The food record form has been tested for face and content validity in a previous study by the same researcher on triathletes (71). The participants completed the food record prior to T1 and T2 in order to monitor dietary intake before and during the event. Research assistants assisted and prompted the subjects before and after T1 and T2 to complete the record for the day. All subjects were trained by the researcher on how to keep a food record. The athletes were required to write down exactly what they ate and drank, and the amount of this, at the time of intake for two days before, the morning of and during T1 and T2.

Portion sizes were either weighed with a small scale (Weigh-Less[®] Food Scale with 400 ml bowl, item code: FS02, capacity: 500 g/18 oz, graduation: 10 g or ¼ oz, www.Weigh-Less.co.za) given to the subjects by the researcher, or quantified using standard household measurements and units, for example one egg, one can of a certain soft drink, or 250 ml of water. The quantities of supplements, such as energy gels- and bars, were recorded as the amounts indicated on the wrapper. Portion sizes of the pre-event meal on the day of the triathlons were weighed. Portion sizes of food and drink consumed during the triathlons were recorded directly after the triathlons. Quantities of sports drinks left over after the race was subtracted from the total amount the athlete took with during the race. Supplements and sports foods consumed were quantified as the portion sizes indicated on the wrapper. The researcher only provided water at the aid stations; therefore, the only sports drink or

supplements ingested were what the athletes brought with them. This was recorded on the food recorded after the race and quantified as explained above.

On the day of each trial, the subjects were instructed to come to the trial in a fasted state, as fasted baseline blood samples needed to be taken in the fasted state. Therefore, they were instructed to bring their scales and food records to the race to include their pre-event meal and their intake during T1 and T2.

The subjects completed the food records up to the end of T1 and T2 and handed these to the research assistants, who checked the records for completeness. Unclear and incomplete records were double-checked and completed with the athletes.

The food record also contained a section on the daily use of supplements (i.e. the brand name, type of supplement, dose, when the supplement was taken and duration/frequency of use), as well as a section to complete on the training the athlete performed on the days of keeping the food record.

Dietary data was analysed with the Food Finder TM3 for Windows[®] software application, Version 1 (Langenhoven 2002). This software application was developed by the Nutritional Intervention Research Unit (NIRU) and Biomedical Informatics Research Division (BIRD) of the South African Medical Research Council (MRC) in collaboration with WAMTechnology CC. The program analyses food intake of individuals or groups of individuals for energy, macro- and micronutrient content (<http://www.foodfinder.mrc.ac.za/>).

Dietary data was analysed separately for habitual caffeine intake; and habitual dietary intake two days before, the morning of and during T1 and T2. If foodstuffs were not available on Food Finder[®], the researcher searched for the nutritional information on the Internet, by looking at brand websites, and added the foodstuff to Food Finder[®]. Dietary supplements were quantified for macronutrients and added to the nutrient analysis.

Habitual and training/racing specific dietary macronutrient intake was compared to nutritional requirements as stated in the latest International Olympic Committee (IOC) consensus documents and where applicable the recommendations of the American Dietetic Association (ADA) and the American College of Sports Medicine (ACSM), and the International Society of Sport Nutrition (ISSN) for total energy; estimated energy availability; and CHO, protein and fat intake for endurance athletes (4, 169, 170, 172, 174, 175, 183, 203).

To calculate estimated energy availability ($_{\text{est}}\text{EA}$), expressed in kcal/kg FFM, the following formula was used (4):

$$_{\text{est}}\text{EA} = (\text{average daily energy intake (kcal)} - \text{average daily exercise energy expenditure (EEE) (kcal)}) / \text{kg FFM}$$

EEE was calculated using the following formulas:

- i) $\text{EEE} = (\text{Metabolic equivalent (MET)} \times \text{minutes exercised}) - \text{activity of daily living}$
- ii) Activity of daily living was calculated by multiplying the minutes exercised with a MET value of 2.3, which represented general daily living (i.e. sitting, walking, cleaning and transport).

A low $_{\text{est}}\text{EA}$ was defined as < 30 kcal/kg FFM and a healthy $_{\text{est}}\text{EA}$ as 45 kcal/kg FFM (4).

The micronutrients that were analysed include habitual iron (mg) and calcium (mg) intake, measured with the food record completed two days before T1 and T2. These results were compared with the Dietary Reference Intakes (DRI) for iron and calcium and cut-off values of $< 67\%$ (inadequate) of the DRI was used to assess adequacy. The ratio of calcium:protein was also calculated and compared to the recommendation of at least 20 mg of calcium for every 1 g of protein (176, 204). Dietary supplements were not quantified for micronutrient content.

3.4.4.3 Body composition and bone mineral density

The height of the subjects was measured using a Seca® 767 Column Scale (Germany) with Height meter. The subjects stood barefoot with minimal clothing (shorts and a light T-shirt). The heels were placed together; the arms were to the side; legs were straight; the shoulders were relaxed; and the head positioned in the Frankfort horizontal plane (i.e. line extending from the most inferior point of the orbital margin to the left tragon, when the head is positioned correctly, the Frankfort horizontal plane is parallel to the fixed headpiece. For length measures, the Frankfort plane is aligned perpendicular to the plane of the measuring table and parallel to the headpiece). The heels, buttocks, scapulae and the back of the head rested against the vertical surface of the stadiometer. The subject inhaled deeply just before the measurement was taken. The breath was held and an erect posture was maintained while the headboard was lowered on the highest point of the head with enough pressure to compress the hair. Measurements were recorded to the nearest 0.1 cm, with the eye level with the headboard to decrease errors of parallax. Hair ornamentation was removed prior to taking the measurement (188).

The weight of the subjects was measured using the same Seca® 767 Column Scale with Height Meter. Each subject was instructed to stand still in the middle of the scale's platform without touching anything and with the body weight equally distributed on both feet. The weight was recorded to the nearest 100 g. The subjects wore light clothing and no shoes (188).

The body mass index (BMI) was interpreted according to the standard guidelines from the World Health Organization (WHO) (205) and calculated with the following formula:

$$\text{BMI (kg/m}^2\text{)} = \text{weight (kg)} / (\text{height (m)} \times \text{height (m)})$$

For all anthropometrical measurements, the average of two measurements was used. If the two consecutive measurements differed significantly, the median of the three measurements were used as per guidelines from the International Society for the Advancement of Kinanthropometry (ISAK).

All subjects underwent a DXA scan in the week before T1 at the Stellenbosch Osteoporosis Centre (Number 1, Oewerpark, Rokewood Avenue, Stellenbosch, 7600), to provide baseline body composition measurements. The subjects were instructed to arrive at the unit in the fasted state (overnight fast). A scan of the lumbar spine, left and right femoral neck and hip as well as whole body bone density and a whole body DXA scan was completed on each subject using a Hologic Discovery QDR-4500 DXA machine (12 December 1997). A qualified radiographer (CA Truebody, National Diploma in Diagnostic Radiography DXA, May 2009) operated the DXA machine. The total whole body reading of x-ray exposure was 0.008 mGy, which can be described as minor, when comparing it with everyday background radiation levels.

The following measurements were recorded from the DXA scan; bone mineral density (BMD) and calculation of T- and Z-scores of the anterior-posterior spine, left femoral neck, left total hip, total hip bilateral average and whole body BMD, as well as bone mineral content (BMC), percentage body fat (%BF), fat mass (kg), lean body mass (LBM) (kg), fat free mass (FFM = LBM + BMC) (kg) and total body mass.

DXA scans were analysed and interpreted by a physician, Dr' G. C. Ellis, Vergelegen Bone Scan (PTY) LTD 7 Arun Place, Sir Lowry's Pass Road, Somerset West, grahamellis@helderbergmedical.co.za).

Various classification systems for the interpretation and classification of BMD exist. The WHO recommends using the T-scores to classify osteopenia and osteoporosis as these scores are compared to average peak BMD. This classification system was used in the current study to evaluate the bone mineral density of post-menopausal women and men over the age of 50, primarily due to the fact that the data from the WHO is predominantly based on Caucasian postmenopausal women (12).

This classification identifies patients as:

- i) Normal, if the T-score is at or above -1.0;
- ii) Osteopenic, if the T-score is between -1.0 and -2.5; or
- iii) Osteoporotic, if the T-score is at or below -2.5

The latest official positions from the International Society for Clinical Densitometry (ISCD) and the American College of Sports Medicine (ACSM) recommend that the classification system from the WHO be used for post-menopausal women and not applied to pre-menopausal women and children (206).

These populations should be classified according to Z-scores, where a Z-score of below -2.0 is defined as “low bone density below the expected range for age” in pre-menopausal women and as “low bone density for chronological age” in children. These recommendations have been adopted by the American Society for Bone and Mineral Research, the International Osteoporosis Foundation and the American Association of Clinical Endocrinologists. The ACSM defines athletes at clinical risk with Z-scores between -1.0 and -2.0, together with a clinical history of fracture as well as nutritional deficiencies, hypo-estrogenism and stress fractures. According to the ACSM, osteoporosis is defined as clinical risk factors as mentioned above with a BMD Z-score of below -2.0 (206).

Therefore, both classification systems were used with the interpretation of the current study's results.

The percentage body fat was interpreted as appropriate for triathletes according to the ACSM and the ADA (183, 207).

3.4.4.4 Training two days before race day

Subjects were instructed to refrain from any exhaustive exercise for 48 hours prior to T1 and T2. If they did any exercise during this time, they were instructed to write it down on the food

record (Appendix 3.14) in the allocated space. The training record prompted the subjects to complete the type of exercise undertaken (for example, running-, or cycling), the duration of the exercise (in minutes) and the rating of self-perceived exertion (on a scale of 1-5, with 1 being “easy” and 5 being “hard”).

3.4.4.5 Caffeine withdrawal

On the day of T1, the subjects were asked to complete a questionnaire administered by the research assistants regarding withdrawal symptoms experienced during the 14 days leading up to T1, in which caffeine-containing foodstuffs were excluded from their diet (Appendix 3.15).

3.4.4.6 Caffeine side-effects

The side effects of caffeine supplementation include: nervousness, restlessness, shakiness, anxiety, heart palpitations, flushing, sleep alteration and headaches. Gastro-intestinal disturbances may also occur, although trials have shown no significant differences in gastroesophageal reflux, gastric pH or gastrointestinal transit time when caffeine supplements are used (44). The incidence of these side effects was recorded during T1 and T2 and is described in the results chapter.

After completion of T1 and T2, the subjects were instructed by the research assistants to complete a questionnaire regarding any side effects of caffeine supplementation that were experienced during T1 or T2 (Appendix 3.16).

3.4.4.7 Hydration status and changes in plasma volume

Serum albumin is produced by the liver and exerts an osmotic effect to maintain water balance between blood and tissue. Serum albumin levels in the present study were determined to detect any changes in plasma volume that might occur due to exercise and to adjust other biochemical parameters accordingly. Serum albumin was measured at baseline, during transition (cycle → run) and at the finish line. Reference values for albumin are 35-50 g/l for both males and females. Details of blood collection are described in section 3.4.2.1.

3.5 Ethics and legal aspects

3.5.1 Ethics approval

The protocol was approved by the Health Research Ethics Committee (HREC) of Stellenbosch University on 1 November 2010. After submission of amendments to the protocol, final approval was granted on 19 April 2011. Annual progress reports were submitted and approved for 2011 and 2012 (M10/08/030) (Appendix 3.17).

3.5.2 Registration with the Medicines Control Council (MCC)

Caffeine supplementation is not intended to treat or cure any medical condition and therefore not regarded as a medicine. It is, instead classified as a nutritional or sport supplement. The researcher, furthermore, used the supplement in healthy athletes at a dosage that did not pose any adverse effects to health. Registration with the MCC was therefore not required.

3.5.3 Informed consent

The researcher recognized autonomy and respect for persons and therefore participation in this study was completely voluntary. The pilot study- and research project were explained in detail by the researcher during the initial interview. Written informed consent was obtained from all subjects who participated in the pilot (Appendix 3.18), as well as in the research project (Appendix 3.19), and subjects were given a copy of the information leaflet and the written informed consent form. The written informed consent form also gave specific information regarding genetic analysis. All subjects also had to sign an indemnity waiver before T1 and T2 (Appendix 3.20).

3.5.4 Anonymity

All subjects were assigned a reference/subject number. The contact details of participants were only used to contact them before, and between T1 and T2. All personal and contact information was deleted from the computer after data collection was completed. All the information gathered during data collection was kept anonymous by using the assigned subject number. Only photos of subjects who gave consent for their photos to be used in this dissertation as well as future publications and presentations were used.

3.5.5 Incentive

After T2, equal prize money was offered to the first five males and females who completed the triathlons in two age categories (Table 3.10). The finishing times for T1 and T2 were combined to determine the winners in each age category.

Prize money is commonly awarded in all triathlon races, but, the amounts awarded can differ significantly between races and there is no known set standard. It is important to note that many athletes race as a source of income, apart from sponsorships. It was not intended that the prize money be a direct incentive for subjects to participate in the study, but rather that it would encourage the athletes to race at the same intensity as they would during a race and that the athletes would compete in both triathlons. The prize money was allocated from the research funds.

Table 3.10 Prize money offered to the triathletes after triathlon 2

	Male	Female
20-39 years	1. R 5 000.00	1. R 5 000.00
	2. R 4 000.00	2. R 4 000.00
	3. R 3 000.00	3. R 3 000.00
	4. R 2 000.00	4. R 2 000.00
	5. R 1 000.00	5. R 1 000.00
40-60 years	1. R 5 000.00	1. R 5 000.00
	2. R 4 000.00	2. R 4 000.00
	3. R 3 000.00	3. R 3 000.00
	4. R 2 000.00	4. R 2 000.00
	5. R 1 000.00	5. R 1 000.00

3.5.6 Insurance

Insurance cover for the clinical field trial was provided by Stellenbosch University (Appendix 3.21).

3.5.7 Funding

This study was supported by grants from the Harry Crossley Foundation; Subcommittee C of the Faculty of Health Sciences, Stellenbosch University; a National Research Foundation PhD scholarship; the Department of Physiology, Stellenbosch University; as well as the Mellon Early Research Career (MERC) Development Program. None of the funders had any vested interest in the outcome of the project and therefore there is no conflict of interest to be declared.

3.5.8 Dissemination of results

Each subject received a confidential report directly from the Osteoporosis clinic regarding the DXA scans. The researcher also sent a summary of the main research findings to the subjects.

Results from this dissertation will be submitted for publication as three to four original research articles in accredited scientific peer-reviewed journals. Journals considered by the researcher includes; Medicine and Science in Sports and Exercise, the International Journal of Sports Medicine, the International Journal of Sports Nutrition or the International Journal of Sports Nutrition and Exercise Metabolism, Current Sports Medicine Reports or the Journal of Applied Physiology.

The researcher plans on submitting part or all of this original research for presentation at the American College of Sports Medicine (ACSM) 60th Annual Meeting and 4th World Congress on Exercise is Medicine® (May 28-June 1, 2013, Indianapolis, Indiana, USA). Further results emanating from this research may also be submitted for oral or poster presentation at other National and International Congresses.

3.6 Statistical analysis

Statistical analysis was completed with the assistance of a biostatistician from the Centre for Statistical Consultation (CSC), Stellenbosch University.

3.6.1 Computer programs

Data capturing and statistical analysis programs used included StatSoft, Statistica® (version 10) and Microsoft Excel (2010) for Windows 7®.

3.6.2 Descriptive statistics

Descriptive statistics in the form of the mean \pm standard deviation (SD)/standard error (SE) for continuous data or data that is normally distributed was used. Nonparametric statistics were used for ordinal/categorical data and for data that was not normally distributed. The data that is not normally distributed is presented as the median and quartile range, or the percentage of the total population.

Some key assumptions made with parametric statistics are that data is normally distributed and that independent, unbiased samples with equal variances are used.

3.6.3 Comparing data between T1 and T2

3.6.3.1 Normally distributed / continuous data

To test the difference between two independent or two dependent groups, the t-test for independent and t-test for dependent samples were used respectively. To test the difference between multiple independent groups, analysis of variance (ANOVA) or multivariate analysis of variance (MANOVA), was performed. The difference between multiple dependent groups was assessed using repeated measures analysis of variance (RMANOVA).

Relationships between variables were tested with the correlation coefficient.

3.6.3.2 Ordinal and categorical and data that is not normally distributed

For ordinal-, and categorical data, as well as data that is not normally distributed, the following non-parametric inferential statistical tests were used:

- i) The Mann-Whitney U test was used to test differences between independent groups;
- ii) Wilcoxon's matched pairs test was used to test the difference between dependent groups;
- iii) McNemar's Chi-square test was used when variables of interest were dichotomous in nature; and
- iv) The Spearman R test was used to test relationships between variables. If these variables of interest were categorical in nature, the Chi-squared test was used.

3.6.3.3 Comparing data between caffeine and placebo groups

The study design was a double-blind, randomized cross-over controlled clinical field trial, in which each subject was his/her own case and control. To compare data between the caffeine and placebo groups, variance estimation, precision and comparison (VEPAC) in STATISTICA® was used to do analysis of variance (ANOVA) with restricted maximum likelihood (REML). This allowed for the respondent to be nested within the treatment group (caffeine received during T1 or T2) and further analysis with the respondent group nested within gender. This approach allowed for the identification of a carry-over effect from whether or not the subject received caffeine during T1 or T2 as each participant was his/her own case (caffeine) and control (placebo). The researcher did not expect a carry-over effect to be present as the washout period between the two trials was 14 days and was deemed adequate in eliminating any possible carry-over effect.

To test differences between ordinal data obtained from the caffeine and placebo groups, the maximum likelihood (M-L) chi-squared test was used.

3.6.3.4 Influence of variables on triathlon performance

To determine the influence of various factors/parameters on triathlon performance, the researcher performed a factorial ANOVA with VEPAC of the overall time to complete the triathlon, with factors group and caffeine and then gender and caffeine, with respondents nested firstly in group and then nested in gender. To investigate the influence of the various factors, these variables were one by one entered as covariates in a similar analysis of covariance (ANACOVA) with factors group and caffeine, and then gender and caffeine.

3.6.3.5 Display of results (tables/figures)

Tables are presented as the mean \pm SD for data that is normally distributed/continuous data. For data that is not normally distributed, the median or the percentage of the total population (frequency) is displayed. Relevant inferential statistics are displayed below each table.

Graphs are displayed as least-square (LS) means and vertical bars denote 95% confidence intervals. Relevant inferential statistics are displayed below each graph.

Statistical significance was set at $p < 0.05^*$ and is indicated with an * where relevant. Values that were statistically significant from each other are marked with ^a and ^b.

CHAPTER 4: RESULTS

4.1 Demographic information

The total number of subjects who participated in both T1 and T2 were $N = 26$, of which 54% ($N_m = 14$) were male and 46% ($N_f = 12$) were female. The mean age was 37.8 (± 10.6) years, with the mean age of the males being 38.4 (± 10.0) years and the mean age of the females being 37.2 (± 11.6) years. All the subjects were Caucasian.

The age distribution of the group was as follows:

- i) 18-29 years (31% of the sample size, $N = 8$);
- ii) 30-40 years (19%, $N = 5$);
- iii) 40-50 years (31%, $N = 8$); and
- iv) 50-60 years (19%, $N = 5$).

4.2 Ergogenic effect of caffeine supplementation

4.2.1 Plasma caffeine

The researcher performed a RMANOVA with VEPAC on plasma caffeine levels with factors group, caffeine and stage (plasma caffeine levels measured at baseline, during transition (cycle \rightarrow run) and at the finish line) and then gender, caffeine and stage with respondents nested firstly in group ($p = 0.92$) and then in gender ($p = 0.17$).

Baseline plasma caffeine levels were taken 1 hour before caffeine supplementation and therefore caffeine supplementation had no influence on baseline caffeine levels ($p = 0.85$ for all; $p = 0.68$ for males and $p = 0.88$ for females). The plasma caffeine measurements taken during transition (cycle \rightarrow run) ($p = 0.00$ for all; $p = 0.00$ for males and $p = 0.00$ for females) and at the finish line (all $p = 0.00$, male $p = 0.00$, female $p = 0.00$) were significantly increased by caffeine supplementation (Table 4.1).

Table 4.1 Caffeine supplementation and plasma caffeine levels in the caffeine and placebo groups

	Caffeine	Placebo	Caffeine vs. Placebo
	Mean±SD	Mean±SD	p-value
All	N_c = 28	N_p = 28	
Caffeine supplementation (mg)	600.6±91.4		
Baseline (mg/l)	1.3±0.7	1.4±1.4	p = 0.85
Transition (cycle-run) (mg/l)	7.0±3.1	1.3±1.2	p = 0.00*
Finish line (mg/l)	8.6±2.8	1.3±1.2	p = 0.00*
Male	N_{cm} = 14	N_{pm} = 14	
Caffeine supplementation (mg)	652.4±76.7		
Baseline (mg/l)	1.5±0.9	1.7±1.9	p = 0.68
Transition (cycle-run) (mg/l)	6.3±3.5 ^a	1.6±1.6 ^b	p = 0.00*
Finish line (mg/l)	8.1±2.9 ^a	1.6±1.6 ^b	p = 0.00*
Female	N_{cf} = 12	N_{pf} = 12	
Caffeine supplementation (mg)	540.1±68.1		
Baseline (mg/l)	1.1±0.3	1.0±0.0	p = 0.88
Transition (cycle-run) (mg/l)	7.9±2.5 ^a	1.0±0.0 ^b	p = 0.00*
Finish line (mg/l)	9.3±2.5 ^a	1.0±0.0 ^b	p = 0.00*

^a differed significantly from ^b (p < 0.05)

N = total sample; N_m = total male sample; N_f = total female sample; N_c = total caffeine; N_{cm} = total caffeine male; N_{cf} = total caffeine female; N_p = total placebo; N_{pm} = total placebo male; N_{pf} = total placebo female

In the placebo group, caffeine levels in the blood remained unchanged over the course of the event (p > 0.05). However, in the caffeine group, caffeine levels increased significantly throughout the triathlon and increased from baseline to transition (cycle → run) and at the finish line (p = 0.00*) (Figure 4.1). This was apparent in the male and female groups, although no statistically significant difference was found between the plasma caffeine levels of males and females (p = 0.17).

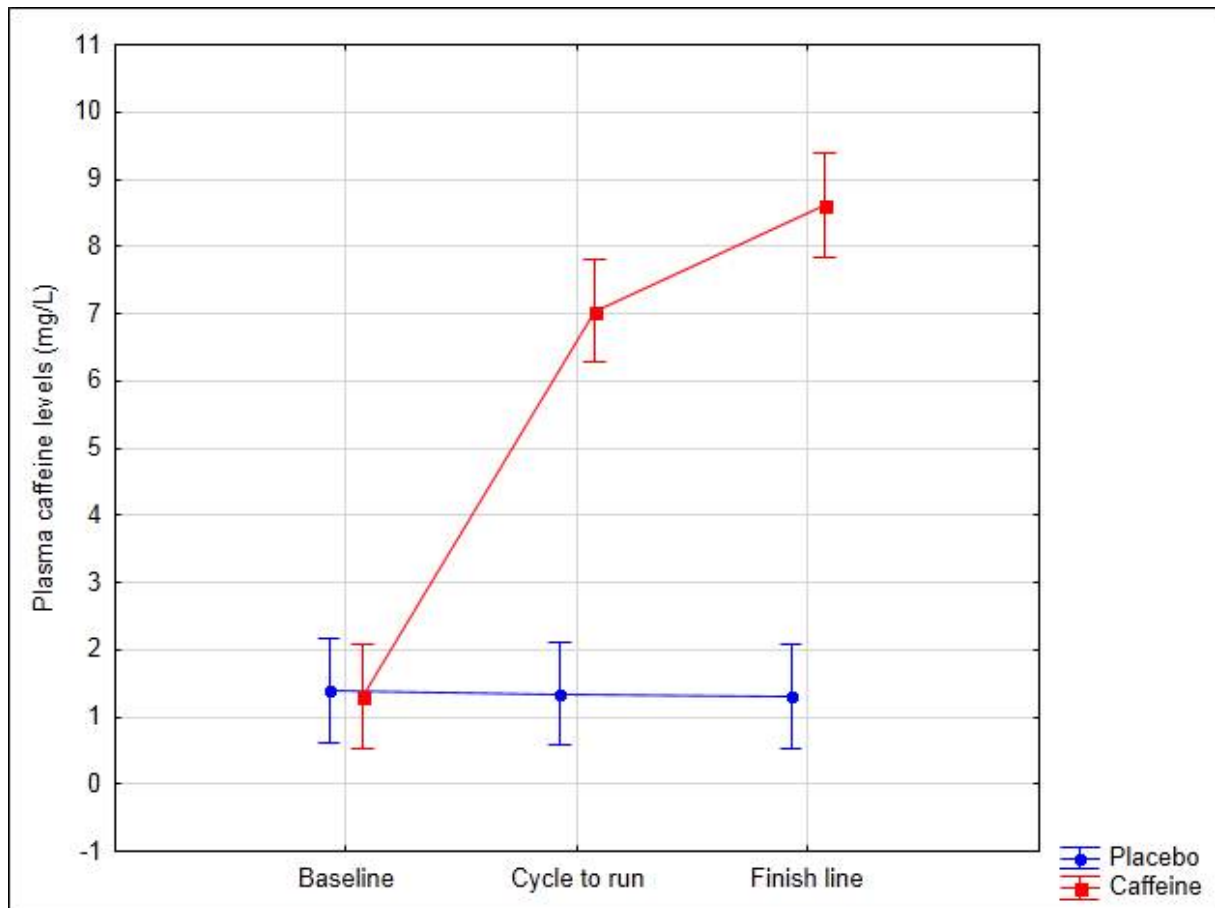


Figure 4.1 Influence of caffeine supplementation on plasma caffeine levels measured at baseline, transition (cycle → run) and at the finish line in the caffeine and placebo groups (Supplementation effect: $p = 0.00^*$)

4.2.2 Triathlon performance

The researcher performed an ANOVA with VEPAC of the time taken to complete the swim (minutes), cycle (minutes), run (minutes), medical (minutes), overall with medical (minutes) and overall time to complete the triathlon without medical (minutes) respectively with factors group and caffeine and then gender and caffeine with respondents nested firstly in group and then nested in gender. Although not always statistically significant, there was a definite trend toward faster overall times and individual sections of the triathlon in the caffeine group compared to the placebo group; this was also evident from the percentage reduction in time that was calculated, as described below.

Caffeine supplementation had the most profound effect on the swim time, which is the first section of the triathlon. There was a 3.7% reduction in the time taken to complete the swim in all subjects (33.5 ± 7.0 vs. 34.8 ± 8.1) ($p = 0.05^*$), a 4.5% reduction in the male subjects (32.2 ± 4.6 vs. 33.7 ± 6.3) ($p = 0.07$) and a 2.8% decrease in the female group (35.0 ± 9.0 vs.

36.0±9.9) ($p = 0.29$). This effect was also observed in the overall time to complete the triathlon in all subjects, with a 1.3% reduction in the overall time to complete the triathlon (149.6±19.8 vs. 151.5±18.6) ($p = 0.02^*$), a 1.7% decrease in the male subjects (142.6±11.9 vs. 145.0±12.7) ($p = 0.04^*$) and a 0.9% reduction in the female group (157.8±24.3 vs. 159.2±21.9) ($p = 0.29$) (Table 4.2). Twenty of the triathletes (20/26) had decreased overall time to complete the triathlons, with only six (6/26) displaying either no or limited improvement in performance time (Figure 4.2).

Table 4.2 Time to complete the various sections of the triathlon

	All	Caffeine	Placebo	Caffeine vs. Placebo	% ↓
	Mean±SD	Mean±SD	Mean±SD	p-value	
All	$N = 52$	$N_c = 26$	$N_p = 26$		
Swim (minutes)	34.1±7.5	33.5±7.0 ^a	34.8±8.1 ^b	$p = 0.05^*$	3.7 %
Cycle (minutes)	71.0±7.2	70.6±7.9	71.4±6.5	$p = 0.33$	1.1%
Run with medical (minutes)	47.9±6.4	47.6±6.6	48.1±6.4	$p = 0.18$	
Medical* (minutes)	2.1±0.9	2.0±0.7	2.2±1.0	$p = 0.43$	
Run without medical (minutes)	45.8±6.3	45.6±6.4	45.9±6.3	$p = 0.24$	0.7%
Overall with medical (minutes)	152.7±19.1	151.6±19.8 ^a	153.8±18.7 ^b	$p = 0.01^*$	
Overall without medical (minutes)	150.6±19.1	149.6±19.8 ^a	151.5±18.6 ^b	$p = 0.02^*$	1.3%
Males	$N_m = 28$	$N_{cm} = 14$	$N_{pm} = 14$		
Swim (minutes)	32.9±5.5	32.2±4.6	33.7±6.3	$p = 0.07$	4.5%
Cycle (minutes)	67.8±4.7	67.2±4.1	68.4±5.3	$p = 0.29$	1.8%
Run with medical (minutes)	45.6±5.0	45.2±5.0	46.1±5.1	$p = 0.10$	
Medical* (minutes)	2.1±0.9	1.9±0.6	2.2±1.2	$p = 0.29$	
Run without medical (minutes)	43.6±4.9	43.3±4.9	43.8±5.0	$p = 0.22$	1.1%
Overall with medical (minutes)	145.9±12.3	144.5±12.0 ^a	147.2±12.9 ^b	$p = 0.02^*$	
Overall without medical (minutes)	143.8±12.1	142.6±11.9 ^a	145.0±12.7 ^b	$p = 0.04^*$	1.7%
Females	$N_f = 24$	$N_{cf} = 12$	$N_{pf} = 12$		
Swim (minutes)	35.5±9.2	35.0±9.0	36.0±9.9	$p = 0.29$	2.8%
Cycle (minutes)	74.7±7.9	74.5±9.5	74.9±6.2	$p = 0.78$	0.5%
Run with medical (minutes)	50.5±6.9	50.4±7.2	50.6±7.0	$p = 0.78$	
Medical* (minutes)	2.2±0.8	2.2±0.9	2.2±0.8	$p = 0.94$	
Run without medical (minutes)	48.3±6.9	48.2±7.1	48.4±7.0	$p = 0.77$	0.4%
Overall with medical (minutes)	160.7±22.6	159.9±24.2	161.4±21.9	$p = 0.22$	
Overall without medical (minutes)	158.5±22.7	157.8±24.3	159.2±21.9	$p = 0.24$	0.9%

^a differed significantly from ^b ($p < 0.05$)

*Medical: time taken to draw blood sample during transition from cycle-run

N = total sample; N_m = total male sample; N_f = total female sample; N_c = total caffeine; N_{cm} = total caffeine male; N_{cf} = total caffeine female; N_p = total placebo; N_{pm} = total placebo male; N_{pf} = total placebo female

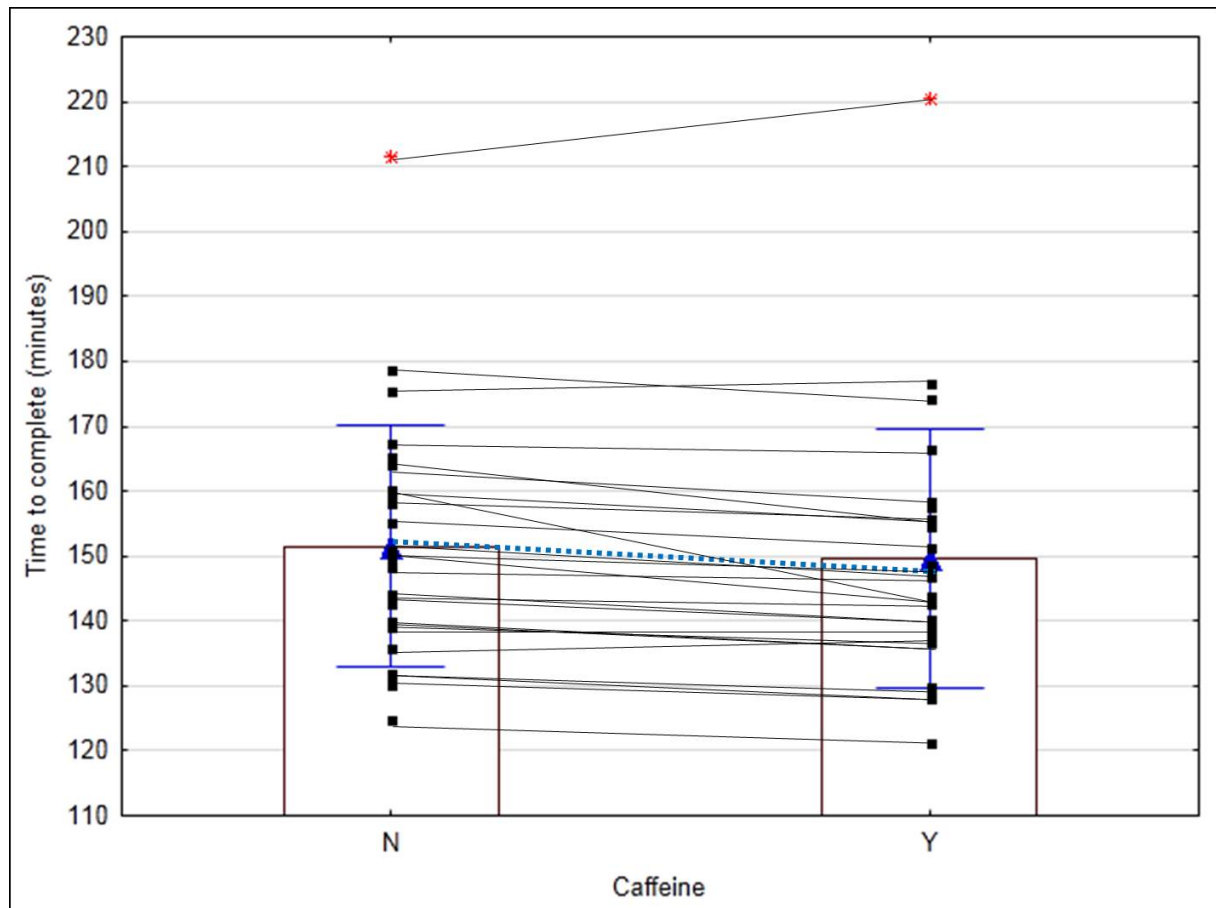


Figure 4.2 Overall time to complete the triathlons in the caffeine and placebo groups (Supplementation effect: $p = 0.02^*$)

*indicates outliers, Δ - - - - indicates mean and SD

4.2.3 Rating of perceived exertion (RPE)

4.2.3.1 RPE in the caffeine and placebo groups

Ratings of perceived exertion were measured with the Borg scale RPE during transition (swim \rightarrow cycle), transition (cycle \rightarrow run) and again at the finish line. There were no significant differences in the RPE measured at all the time points in the caffeine and placebo groups, irrespective of gender. There was, however a tendency towards slightly decreased RPE values in the caffeine group compared to the placebo group, but this was not statistically significant (Table 4.3).

Table 4.3 Ratings of perceived exertion in the caffeine and placebo groups

	All	Caffeine	Placebo	Caffeine vs. Placebo
	Mean±SD	Mean±SD	Mean±SD	p-value
All	N = 52	N_c = 26	N_p = 26	
Transition (swim-cycle)	15.3±1.7	15.1±1.7	15.4±1.8	p = 0.45
Transition (cycle-run)	15.8±1.7	15.8±1.7	15.8±1.8	p = 0.93
Finish line	17.4±2.2	17.2±2.2	17.5±2.3	p = 0.50
Males	N_m = 28	N_{cm} = 14	N_{pm} = 14	
Transition (swim-cycle)	15.8±1.4	15.7±1.5	15.8±1.4	p = 0.91
Transition (cycle-run)	16.0±1.6	16.1±1.5	15.9±1.8	p = 0.65
Finish line	17.7±2.3	17.5±2.5	17.9±2.2	p = 0.57
Females	N_f = 24	N_{cf} = 12	N_{pf} = 12	
Transition (swim-cycle)	14.7±1.9	14.3±1.6	15.0±2.2	p = 0.33
Transition (cycle-run)	15.5±1.8	15.3±1.8	15.8±1.8	p = 0.54
Finish line	17.0±2.1	16.9±2.0	17.2±2.4	p = 0.71

N = total sample; N_m = total male sample; N_f = total female sample; N_c = total caffeine; N_{cm} = total caffeine male; N_{cf} = total caffeine female; N_p = total placebo; N_{pm} = total placebo male; N_{pf} = total placebo female

The researcher performed a RMANOVA with VEPAC of the RPE with factors group, caffeine and stage (transition (swim → cycle), transition (cycle → run) and finish line) and then gender, caffeine and stage; with respondents nested firstly in group (p = 0.95) and then in gender (p = 0.80).

There were no significant differences in the caffeine group between RPE measured during transition (swim → cycle) and during transition (cycle → run) in the combined male and female group (p = 0.13), and male (p = 0.49) and female (p = 0.14) groups. There were also no significant differences in the placebo group between RPE measured during transition (swim → cycle) and during transition (cycle → run) in the combined male and female group (p = 0.40), and male (p = 0.91) and female (p = 0.27) groups.

There were however significant increases in RPE measured during transition (swim → cycle) and at the finish line in the combined male and female group (p = 0.00*), and male (p = 0.00*) and female (p = 0.00*) groups and also between the RPE measured during transition (cycle → run) and at the finish line in the combined male and female group (p = 0.00*), and male (p = 0.03*) and female (p = 0.02*) groups with caffeine supplementation.

There was also a significant increase in RPE measured during transition (swim → cycle) and at the finish line in the combined male and female group (p = 0.00*), and male (p = 0.00*) and female (p = 0.00*) groups and also between the RPE measured during transition (cycle

→ run) and at the finish line in the combined male and female group ($p = 0.00^*$), and male ($p = 0.00^*$) and female ($p = 0.04^*$) groups in the placebo group.

Although there was a trend toward lower RPE values in the caffeine vs. placebo group, this was not statistically significant ($p = 0.87$) (Figure 4.3).

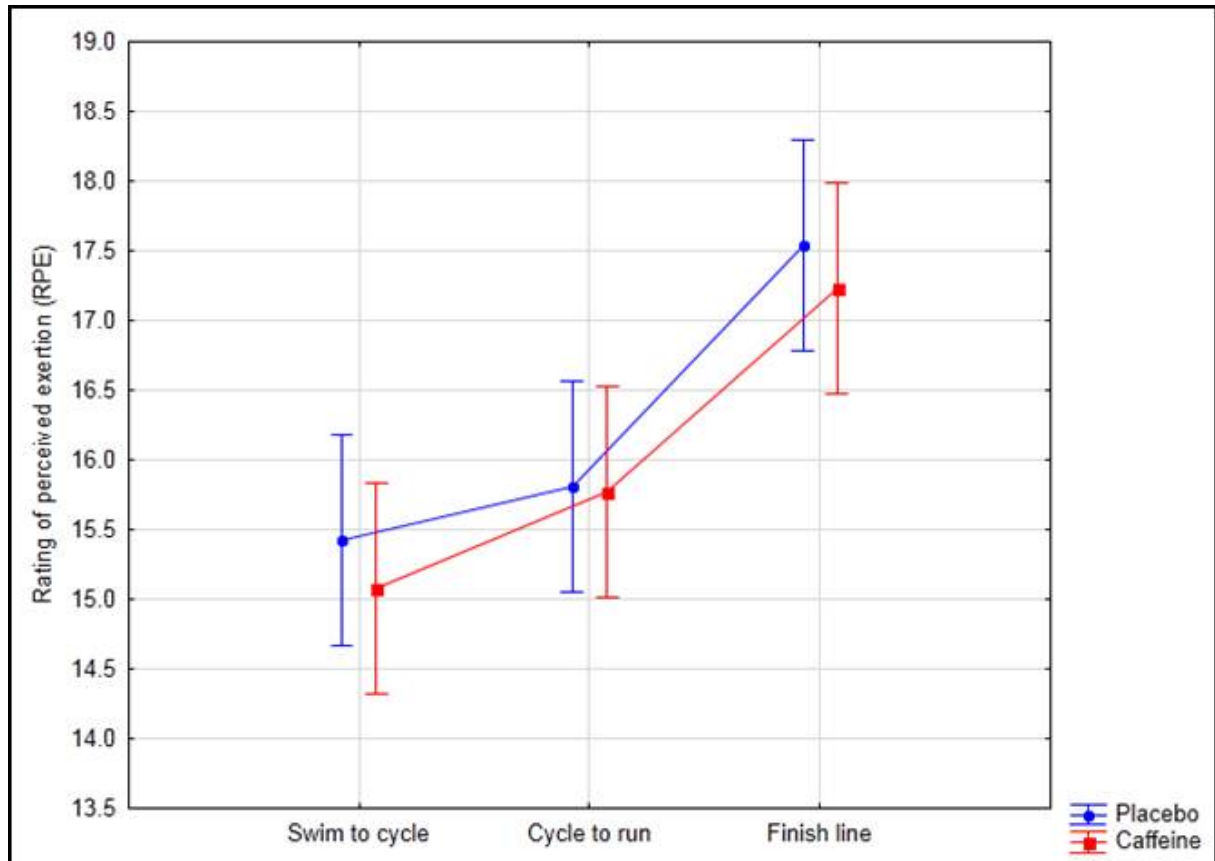


Figure 4.3 RPE measured at various time points in the caffeine and placebo groups (Supplementation effect: $p = 0.87$, significant difference between time points: $p = 0.00^*$)

4.2.3.2 Influence of RPE on triathlon performance

The researcher performed a factorial ANOVA with VEPAC of the overall time to complete the triathlon, the cycling and running times respectively, with factors group and caffeine and then gender and caffeine, with respondents nested firstly in group and then nested in gender. To investigate the influence of RPE measured during transition (swim → cycle), transition (cycle → run) and at the finish line respectively, these variables were one by one entered as covariates in a similar analysis of covariance with factors (group and caffeine, and then gender and caffeine), as described above.

The introduction of RPE measured during transition (swim → cycle) ($p = 0.35$ with group and $p = 0.49$ with gender), transition (cycle → run) ($p = 0.34$ with group and $p = 0.34$ with gender) and at the finish line ($p = 0.13$ with group and $p = 0.12$ with gender) as a covariate had no significant effect on the overall time to complete the triathlon.

The introduction of RPE measured during transition (swim → cycle) as a covariate had no significant effect on the time to complete the cycling section of the triathlon ($p = 0.43$ with group and $p = 0.97$ with gender).

The introduction of RPE measured during transition (cycle → run) as a covariate also had no significant effect on the time to complete the running section of the triathlon ($p = 0.93$ with group and $p = 0.74$ with gender).

4.2.3.3 Influence of RPE measured during transition (swim → cycle), transition (cycle → run) and at the finish line on plasma cortisol levels

The researcher performed a factorial ANOVA with VEPAC of the plasma cortisol levels measured at the finish line, with factors group and caffeine and then gender and caffeine, with respondents nested firstly in group and then nested in gender. To investigate the influence of RPE measured during transition (swim → cycle), transition (cycle → run) and at the finish line respectively, these variables were one by one entered as covariates in a similar analysis of covariance with factors (group and caffeine, and then gender and caffeine), as described above.

The introduction of RPE measured during transition (swim → cycle) ($p = 0.46$), transition (cycle → run) ($p = 0.50$) and at the finish line ($p = 0.86$) as a covariate had no significant effect on plasma cortisol levels measured at the finish line.

4.2.4 Mood state

The researcher performed a RMANOVA with VEPAC on the total POMS score with factors group, caffeine and stage (the week before, at baseline and at the finish line); and then gender, caffeine and stage; with respondents nested firstly in group ($p = 0.92$) and then nested in gender ($p = 0.87$). Caffeine supplementation made no difference in the total POMS score measured the week before, at baseline or at the finish line in the caffeine and placebo groups ($p = 0.72$), irrespective of gender ($p = 0.87$) (Figure 4.4).

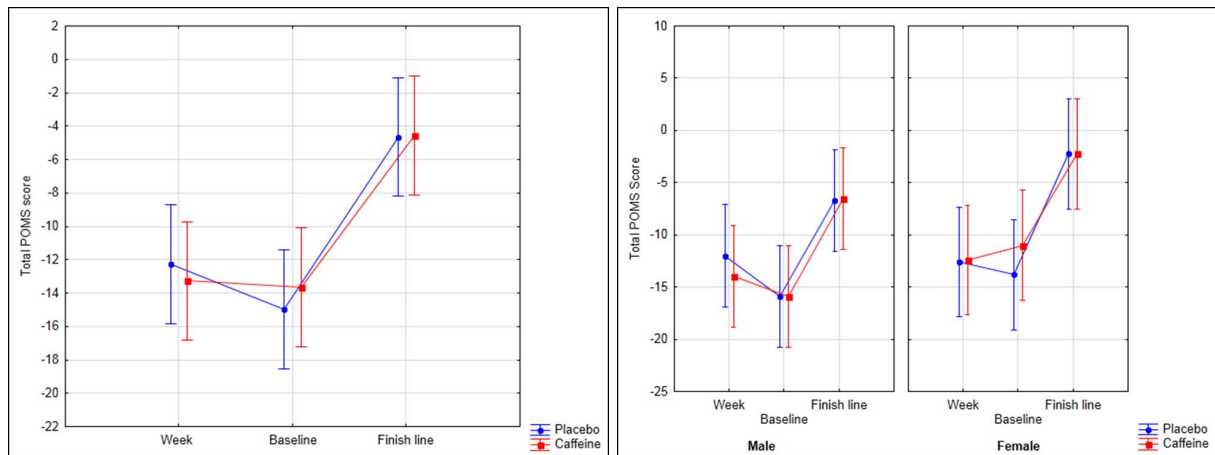


Figure 4.4 Total POMS scores measured the week before, at baseline and at the finish line in the caffeine and placebo groups (Supplementation effect: $p = 0.72$) and according to gender (Gender effect: $p = 0.87$).

The researcher performed a RMANOVA with VEPAC with factors group, caffeine and stage (the week before, at baseline and at the finish line); and then gender, caffeine and stage; with i) POMS tension score nested firstly in group ($p = 0.32$) and then gender ($p = 0.90$), ii) POMS vigour score nested firstly in group ($p = 0.90$) and then gender ($p = 1.00$) and iii) POMS fatigue score, nested firstly in group ($p = 0.77$) and then gender ($p = 0.55$).

Caffeine supplementation made no difference in the differential POMS scores measured the week before, at baseline or at the finish line in the caffeine and placebo groups for i) POMS tension score ($p = 0.19$), irrespective of gender ($p = 0.90$), ii) POMS vigour score ($p = 0.64$), irrespective of gender ($p = 1.00$) and POMS fatigue score ($p = 0.32$), irrespective of gender ($p = 0.55$) (Table 4.4)

Table 4.4 POMS score between caffeine and placebo groups

	All		Male		Female		Caffeine vs. Placebo	Male vs. Female
	Caffeine (N _c = 26)	Placebo (N _p = 26)	Caffeine (N _{mc} = 14)	Placebo (N _{mp} = 14)	Caffeine (N _{fc} = 12)	Placebo (N _{fp} = 12)		
Week before	Mean±SD	Mean±SD	Mean±SD	Mean±SD	Mean±SD	Mean±SD	p-value	p-value
Total score	-13.3±8.5	-12.3±7.6	-14.0±8.0	-12.0±8.1	-12.4±9.4	-12.6±7.4	p = 0.72	p = 0.87
Tension	-2.7±2.0	-2.5±1.8	-2.8±1.6	-2.8±1.6	-2.6±2.4	-2.2±2.0	p = 0.19	p = 0.90
Vigor	17.0±4.2	17.2±3.2	17.6±3.7	17.3±3.6	16.2±4.7	17.2±2.9	p = 0.64	p = 1.00
Fatigue	6.4±5.6	7.5±5.9	6.4±5.7	8.1±6.3	6.3±5.7	6.8±5.5	p = 0.32	p = 0.55
Baseline	Mean±SD	Mean±SD	Mean±SD	Mean±SD	Mean±SD	Mean±SD	p-value	p-value
Total score	-13.7±9.1	-15.0±8.5	-15.9±6.7	-15.9±8.4	-11.0±11.0	-13.8±8.8	p = 0.72	p = 0.87
Tension	-2.6±2.6	-2.5±2.2	-2.9±2.1	-2.8±1.8	-2.3±3.2	-2.2±2.6	p = 0.19	p = 0.90
Vigour	16.0±5.6	16.1±5.4	18.1±4.4	17.5±4.7	13.5±6.1	14.4±5.9	p = 0.64	p = 1.00
Fatigue	4.9±5.2	3.6±4.1	5.0±5.3	4.4±5.1	4.8±5.3	2.8±2.4	p = 0.32	p = 0.55
Finish line	Mean±SD	Mean±SD	Mean±SD	Mean±SD	Mean±SD	Mean±SD	p-value	p-value
Total score	-4.6±11.7	-4.7±9.2	-6.6±13.0	-6.7±10.7	-2.3±10.1	-2.3±6.5	p = 0.72	p = 0.87
Tension	-0.6±2.1	-1.7±2.3	-0.6±2.1	-1.6±1.6	-0.5±2.2	-1.8±3.0	p = 0.19	p = 0.90
Vigour	15.5±6.6	14.4±6.0	18.3±6.6	16.6±6.0	12.3±5.1	11.8±5.1	p = 0.64	p = 1.00
Fatigue	11.5±5.9	11.5±5.3	12.4±5.6	11.5±5.9	10.6±6.4	11.4±4.9	p = 0.32	p = 0.55

^a differed significantly from ^b (p < 0.05)N = total sample; N_m = total male sample; N_f = total female sample; N_c = total caffeine; N_{cm} = total caffeine male; N_{cf} = total caffeine female; N_p = total placebo; N_{pm} = total placebo male; N_{pf} = total placebo female

4.3 Parameters that could in part explain the ergogenicity of caffeine supplementation

4.3.1 Endocrine-stress response

Serum cortisol, DHEAs, prolactin and testosterone levels were measured at baseline and at the finish line in the caffeine and placebo groups (Table 4.5).

Table 4.5 Levels of various endocrine-stress hormones in the caffeine and placebo groups

	All	Caffeine	Placebo	Caffeine vs. Placebo
	Mean±SD	Mean±SD	Mean±SD	p-value
All	N = 52	N_c = 26	N_p = 26	
Cortisol				
Baseline (nmol/l)	569.9±140.6	570.5±142.6	569.3±141.5	p = 0.90
Finish line (nmol/l)	604.0±193.1	664.9±199.7 ^a	543.0±168.7 ^b	p = 0.00*
DHEAS				
Baseline (umol/l)	6.6±3.3	6.7±3.4	6.6±3.3	p = 0.52
Finish line (umol/l)	7.8±3.8	7.9±4.1	7.6±3.6	p = 0.16
Prolactin				
Baseline (ug/l)	11.0±4.7	10.8±5.0	11.1±4.5	p = 0.93
Finish line (ug/l)	20.6±12.8	21.8±13.5	19.4±12.2	p = 0.24
Testosterone				
Baseline (nmol/l)	9.8±8.8	9.9±8.9	9.6±8.7	p = 0.59
Finish line (nmol/l)	7.6±6.7	7.7±6.7	7.5±6.8	p = 0.77
Male				
	N_m = 28	N_{cm} = 14	N_{pm} = 14	
Cortisol				
Baseline (nmol/l)	523.4±88.9	511.0±88.2	535.9±91.1	p = 0.50
Finish line (nmol/l)	615.3±185.6	675.4±201.1 ^a	555.3±152.8 ^b	p = 0.00*
DHEAS				
Baseline (umol/l)	8.3±3.4	8.4±3.6 ^a	8.2±3.4 ^b	p = 0.01*
Finish line (umol/l)	9.6±3.9	9.8±4.4	9.4±3.6	p = 0.16
Prolactin				
Baseline (ug/l)	10.2±3.7	9.3±3.5	11.1±3.8	p = 0.50
Finish line (ug/l)	17.3±6.5	18.5±6.5	16.2±6.4	p = 0.23
Testosterone				
Baseline (nmol/l)	16.7±5.5	17.1±5.4	16.4±5.8	p = 0.34
Finish line (nmol/l)	13.1±4.1	13.3±3.8	12.9±4.5	p = 0.70
Female				
	N_f = 24	N_{cf} = 12	N_{pf} = 12	
Cortisol				
Baseline (nmol/l)	624.2±169.9	640.0±165.0	608.4±180.5	p = 0.43
Finish line (nmol/l)	590.8±204.6	652.8±206.2 ^a	528.8±191.5 ^b	p = 0.00*
DHEAS				
Baseline (umol/l)	4.7±1.8	4.7±1.7	4.7±2.0	p = 0.98
Finish line (umol/l)	5.6±2.3	5.7±2.3	5.5±2.4	p = 0.59
Prolactin				
Baseline (ug/l)	11.9±5.6	12.6±6.0	11.2±5.4	p = 0.62
Finish line (ug/l)	24.3±16.9	25.5±18.2	23.1±16.2	p = 0.41
Testosterone				
Baseline (nmol/l)	1.7±2.6	1.6±2.2	1.7±3.0	p = 0.85
Finish line (nmol/l)	1.2±0.6	1.2±0.7	1.2±0.5	p = 0.97

^a differed significantly from ^b (p < 0.05)N = total sample; N_m = total male sample; N_f = total female sample; N_c = total caffeine; N_{cm} = total caffeine male; N_{cf} = total caffeine female; N_p = total placebo; N_{pm} = total placebo male; N_{pf} = total placebo female

The researcher performed a RMANOVA with VEPAC on serum cortisol levels with factors group, caffeine and stage (baseline and finish line) and then gender, caffeine and stage, with

respondents nested firstly in group ($p = 1.00$) and then in gender ($p = 0.50$). Similar analyses were completed for dehydroepiandrosterone sulphate (DHEAs) levels, nested firstly in group ($p = 0.95$) and then in gender ($p = 0.96$); prolactin, nested firstly in group ($p = 0.46$) and then in gender ($p = 0.58$); and testosterone levels, also nested firstly in group ($p = 0.41$) and then in gender ($p = 0.70$).

4.3.1.1 Cortisol levels

Serum cortisol levels measured in the caffeine group increased significantly from baseline to the finish line in all subjects ($p = 0.00^*$) and the male ($p = 0.00^*$) group, but not in the female group ($p = 0.75$). Serum cortisol levels measured in the placebo group, decreased from baseline to the finish line in all subjects ($p = 0.27$) and the female group ($p = 0.05^*$) and increased slightly from baseline to the finish line in the male ($p = 0.60$) group.

There was a significant increase in serum cortisol levels in the caffeine group compared to the placebo groups ($p = 0.00^*$) (Figure 4.5).

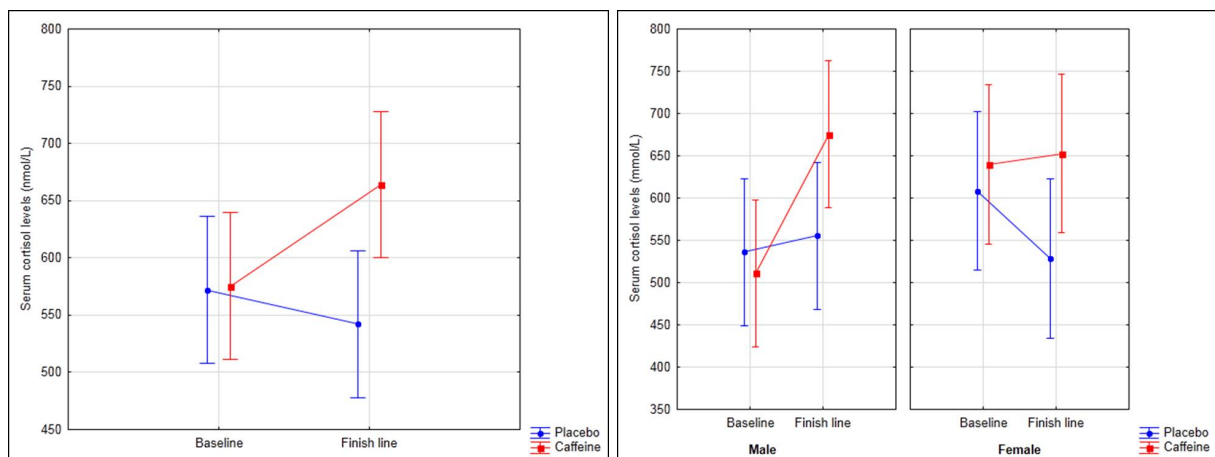


Figure 4.5 Serum cortisol levels measured at baseline and at the finish line in the caffeine and placebo groups (Supplementation effect: $p = 0.00^*$) and according to gender (Gender effect: $p = 0.50$)

There was a significant difference in serum cortisol levels measured at baseline and at the finish line between males and females ($p = 0.00^*$). Males had lower baseline values, which increased at the finish line, whereas females had higher baseline serum cortisol levels that decreased at the finish line (Figure 4.6).

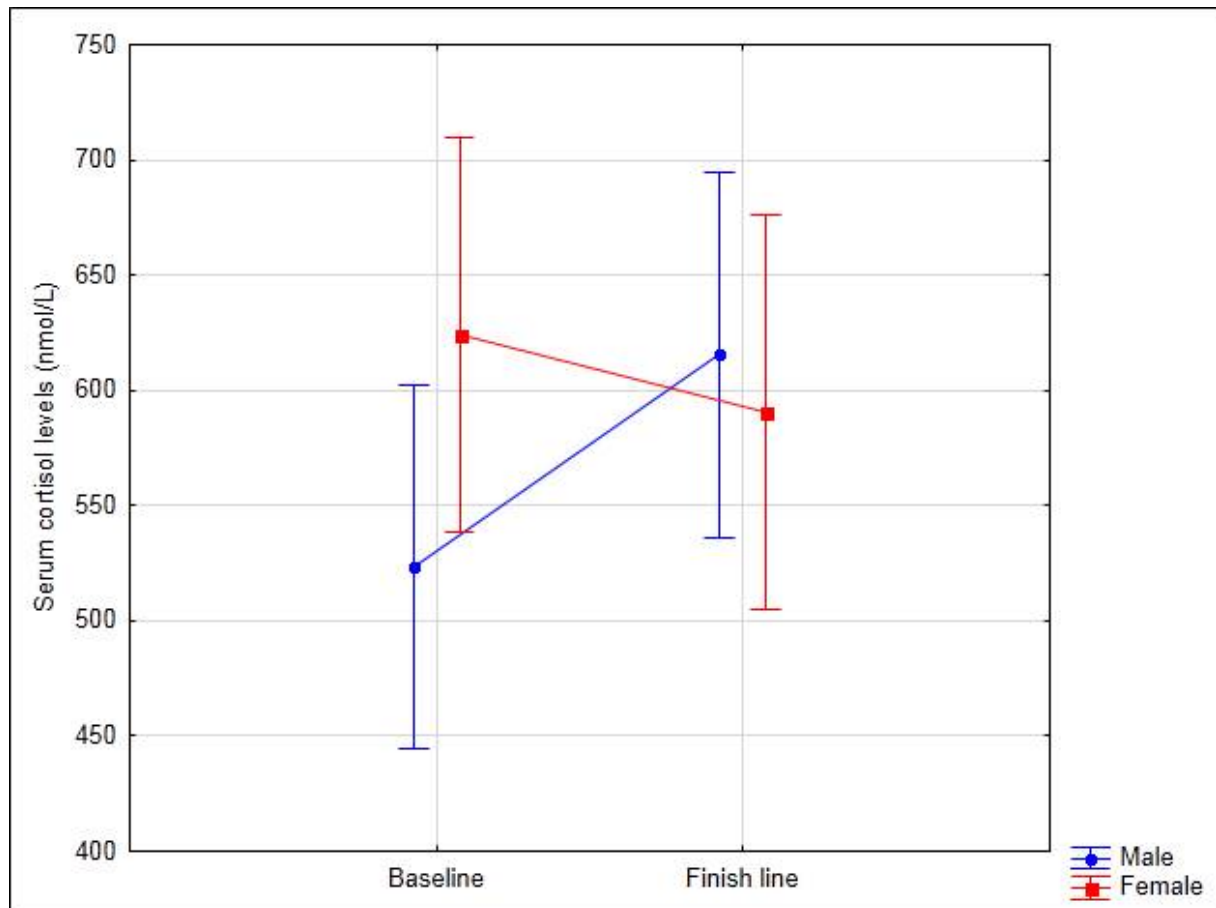


Figure 4.6 Serum cortisol levels measured at baseline and at the finish line in males and females (Gender effect: $p = 0.00^*$)

4.3.1.2 Dehydroepiandrosterone sulphate (DHEAs) levels

DHEAs levels measured in the caffeine group increased significantly from baseline to the finish line in all subjects ($p = 0.00^*$), and male ($p = 0.00^*$) and female ($p = 0.00^*$) groups. DHEAs levels measured in the placebo group were higher in all subjects ($p = 0.00^*$), male ($p = 0.00^*$) and female groups ($p = 0.02^*$). The increase in DHEAs levels was thus not due to caffeine supplementation ($p = 0.58$) and did not differ between males and females ($p = 0.17$) (Figure 4.7).

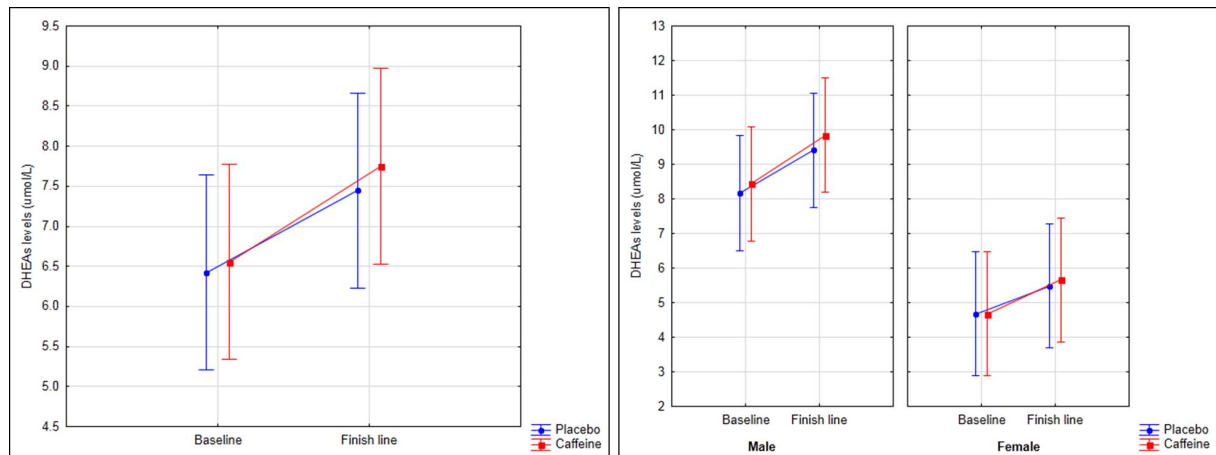


Figure 4.7 DHEAs levels measured at baseline and at the finish line in the caffeine and placebo groups (Supplementation effect: $p = 0.58$) and according to gender (Gender effect: $p = 0.96$)

4.3.1.3 Prolactin levels

Prolactin levels measured in the caffeine group increased significantly from baseline to the finish line in all subjects ($p = 0.00^*$), and male ($p = 0.00^*$) and female groups ($p = 0.00^*$). Prolactin levels measured in the placebo group were also higher in all subjects ($p = 0.00^*$), and male ($p = 0.00^*$) and female groups ($p = 0.00^*$). Therefore, the increase in prolactin levels that was observed was not due to caffeine supplementation ($p = 0.36$), and did not differ between males and females ($p = 0.07$) (Figure 4.8).

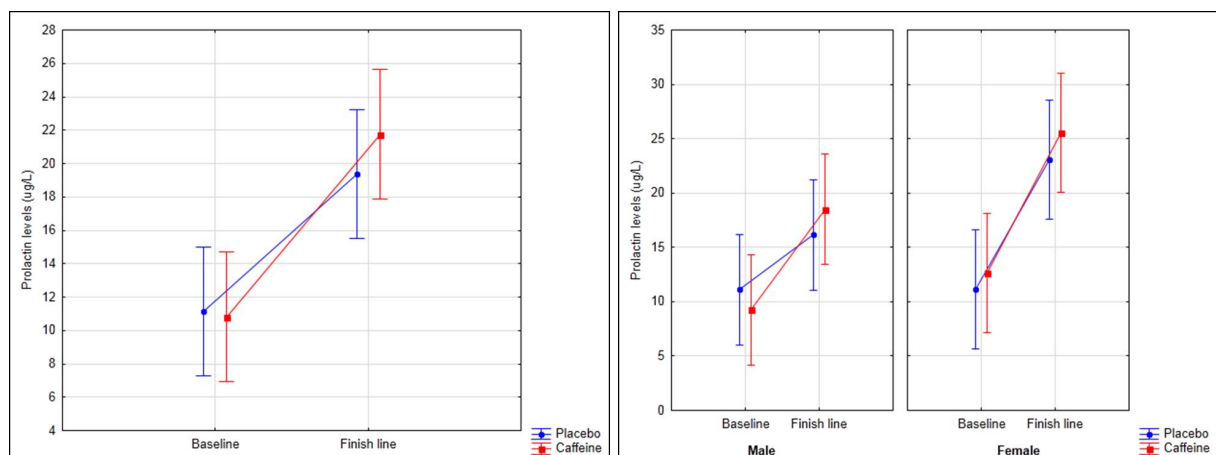


Figure 4.8 Prolactin levels measured at baseline and at the finish line in the caffeine and placebo groups (Supplementation effect: $p = 0.36$) and according to gender (Gender effect: $p = 0.58$)

4.3.1.4 Testosterone levels

Testosterone levels measured in the caffeine group decreased significantly from baseline to the finish line in all subjects ($p = 0.00^*$) and the male ($p = 0.00^*$) group, but remained unchanged in the female group ($p = 0.66$). Similar results were found in the placebo group, with testosterone levels decreasing significantly from baseline to the finish line in all subjects ($p = 0.00^*$) and the male group ($p = 0.00^*$); this was not the case, however, in the female group ($p = 0.51$). Therefore, the change observed in testosterone levels was not due to caffeine supplementation ($p = 0.88$) (Figure 4.9). As expected, males had significantly higher testosterone levels at all the time points, compared to females ($p = 0.00^*$) (Figure 4.10).

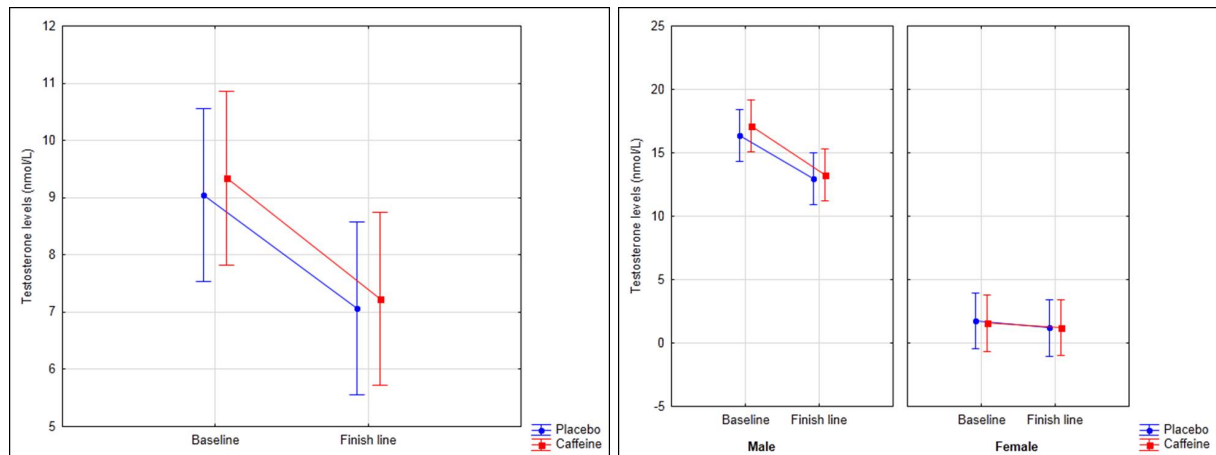


Figure 4.9 Testosterone levels measured at baseline and at the finish line in the caffeine and placebo groups (Supplementation effect: $p = 0.88$) and according to gender (Gender effect: $p = 0.70$)

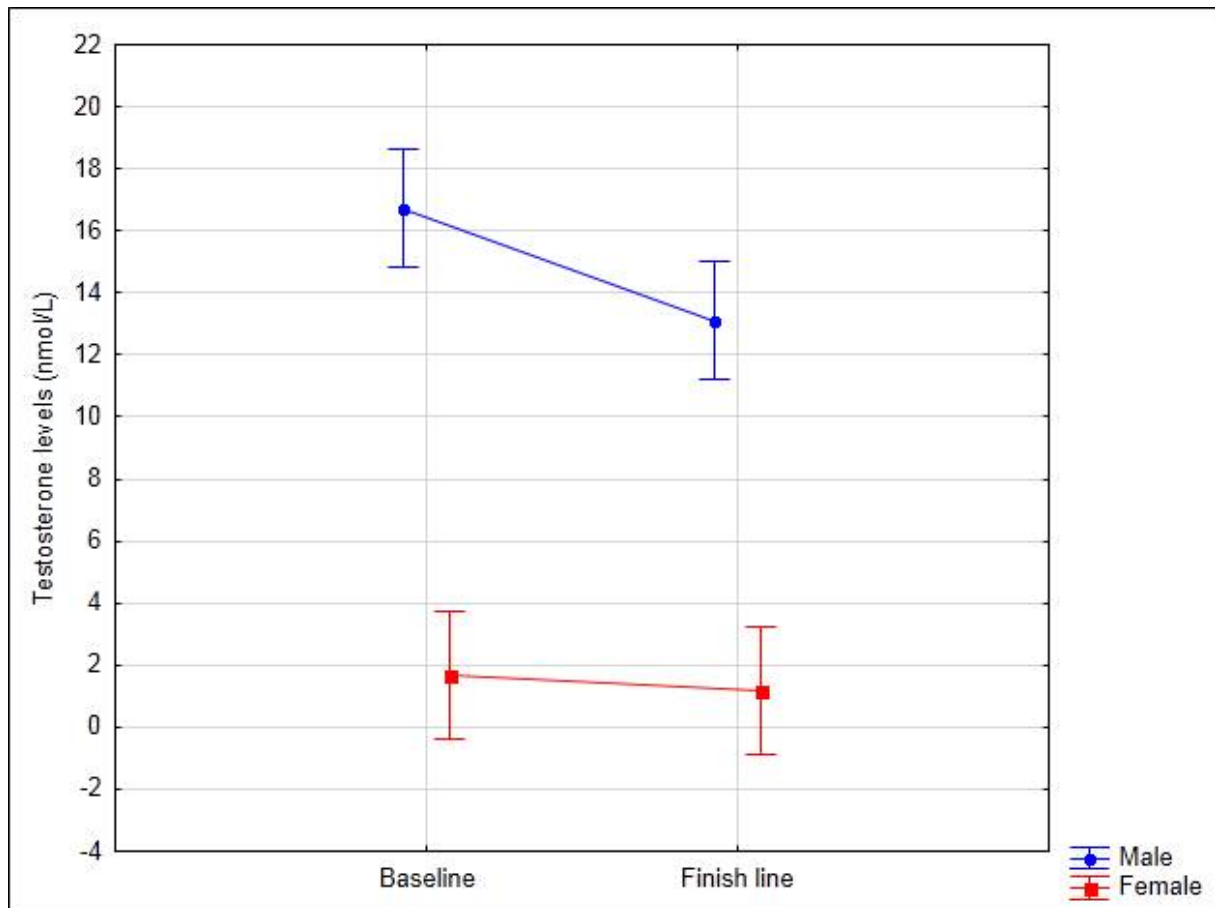


Figure 4.10 Testosterone levels measured at baseline and at the finish line in males and females (Gender effect: $p = 0.00^*$)

4.3.2 Oxidative stress (total and differential white blood cell count)

The researcher performed a RMANOVA with VEPAC on total white blood cell count levels with factors group, caffeine and stage (baseline and finish line) and then gender, caffeine and stage, with respondents nested firstly in group ($p = 0.56$) and then in gender ($p = 0.22$) (Table 4.6).

Table 4.6 Total and differential white blood cell count

	All	Caffeine	Placebo	Caffeine vs. Placebo
	Mean±SD	Mean±SD	Mean±SD	p-value
All	N = 52	N_c = 26	N_p = 26	
White blood cells				
Baseline (X 10 ⁹ /l)	6.2±1.6	6.2±1.8	6.1±1.4	p = 0.88
Finish line (X 10 ⁹ /l)	15.2±3.9	16.1±4.2 ^a	14.2±3.5 ^b	p = 0.01*
Neutrophils				
Baseline	3.2±1.5	3.2±1.7	3.2±1.2	p = 0.95
Finish line	11.4±3.8	12.4±4.1 ^a	11.0±3.3 ^b	p = 0.03*
Baseline (%)	51.0±9.2	50.5±9.6	51.5±8.9	p = 0.54
Finish line (%)	76.3±7.0	75.9±7.6	76.7±6.5	p = 0.51
Lymphocytes				
Baseline	2.2±0.5	2.2±0.5	2.2±0.4	p = 0.73
Finish line	2.5±0.8	2.7±0.8 ^a	2.3±0.7 ^b	p = 0.00*
Baseline (%)	36.9±8.3	37.3±8.9	36.5±7.9	p = 0.57
Finish line (%)	17.2±6.3	17.7±7.0	16.8±5.8	p = 0.48
Monocytes				
Baseline	0.5±0.1	0.5±0.1	0.5±0.1	p = 0.99
Finish line	0.8±0.2	0.9±0.2 ^a	0.8±0.3 ^b	p = 0.03*
Baseline (%)	8.0±1.8	8.0±1.7	8.0±2.0	p = 1.00
Finish line (%)	5.4±1.3	5.4±1.2	5.4±1.5	p = 0.99
Eosinophil				
Baseline	0.2±0.1	0.2±0.1	0.2±0.1	p = 0.35
Finish line	0.0±0.0	0.0±0.1	0.0±0.0	p = 0.60
Baseline (%)	2.9±1.6	3.0±1.8	2.8±1.4	p = 0.43
Finish line (%)	0.3±0.4	0.3±0.5	0.3±0.2	p = 0.75
Basophils				
Baseline	0.1±0.0	0.1±0.0	0.1±0.0	p = 0.53
Finish line	0.1±0.0	0.1±0.0	0.1±0.0	p = 0.54
Baseline (%)	1.2±0.4	1.2±0.5	1.2±0.4	p = 0.39
Finish line (%)	0.8±0.3	0.8±0.3	0.8±0.3	p = 0.34
Male				
	N_m = 28	N_{cm} = 14	N_{pm} = 14	
White blood cells				
Baseline (X 10 ⁹ /l)	6.1±1.3	6.1±1.4	6.1±1.3	p = 0.85
Finish line (X 10 ⁹ /l)	16.1±4.0	17.6±4.2 ^a	14.7±3.4 ^b	p = 0.00*
Neutrophils				
Baseline	2.9±1.0	3.0±1.1	2.9±1.0	p = 0.97
Finish line	12.4±3.8	13.5±4.2 ^a	11.4±3.2 ^b	p = 0.01*
Baseline (%)	47.4±7.2	47.2±7.7	47.5±7.0	p = 0.90
Finish line (%)	76.2±7.0	75.7±7.5	76.7±6.7	p = 0.46
Lymphocytes				
Baseline	2.4±0.4	2.4±0.4	2.4±0.4	p = 0.81
Finish line	2.7±0.9	3.1±0.9 ^a	2.4±0.9 ^b	p = 0.00*
Baseline (%)	40.0±7.5	40.5±8.3	39.5±6.9	p = 0.64
Finish line (%)	17.5±6.5	18.4±7.2	16.7±5.9	p = 0.30
Monocytes				
Baseline	0.5±0.1	0.5±0.2	0.5±0.1	p = 0.74
Finish line	0.9±0.3	0.9±0.3	0.8±0.3	p = 0.20
Baseline (%)	8.4±1.8	8.2±1.6	8.6±2.0	p = 0.19

	All	Caffeine	Placebo	Caffeine vs. Placebo
	Mean±SD	Mean±SD	Mean±SD	p-value
Finish line (%)	5.3±1.4	5.0±1.0	5.6±1.6	p = 0.18
Eosinophil				
Baseline	0.2±0.1	0.2±0.1	0.2±0.1	p = 0.83
Finish line	0.0±0.0	0.0±0.1	0.0±0.0	p = 0.71
Baseline (%)	3.1±1.4	3.0±1.5	3.1±1.2	p = 0.86
Finish line (%)	0.2±0.2	0.2±0.2	0.2±0.2	p = 0.86
Basophils				
Baseline	0.1±0.0	0.1±0.0	0.1±0.0	p = 0.32
Finish line	0.1±0.0	0.1±0.0	0.1±0.0	p = 0.45
Baseline (%)	1.2±0.4	1.1±0.4	1.2±0.4	p = 0.14
Finish line (%)	0.8±0.3	0.7±0.2	0.8±0.3	p = 0.39
Female				
	N_f = 24	N_{cf} = 12	N_{pf} = 12	
White blood cells				
Baseline (X 10 ⁹ /l)	6.2±1.9	6.3±2.3	6.1±1.6	p = 0.86
Finish line (X 10 ⁹ /l)	14.1±3.6	14.5±3.8	13.7±3.6	p = 0.35
Neutrophils				
Baseline	3.6±1.8	3.6±2.2	3.5±1.4	p = 0.95
Finish line	11.0±3.6	11.3±3.9	10.7±3.5	p = 0.48
Baseline (%)	55.2±9.5	54.4±10.4	56.1±8.8	p = 0.47
Finish line (%)	76.4±7.2	76.1±8.1	76.6±6.6	p = 0.85
Lymphocytes				
Baseline	2.0±0.4	2.0±0.5	1.9±0.4	p = 0.79
Finish line	2.2±0.5	2.3±0.6	2.2±0.4	p = 0.54
Baseline (%)	33.2±7.9	33.6±8.5	32.9±7.7	p = 0.73
Finish line (%)	17.0±6.3	17.0±6.9	17.0±6.0	p = 0.99
Monocytes				
Baseline	0.5±0.1	0.5±0.1	0.4±0.2	p = 0.75
Finish line	0.8±0.2	0.8±0.2	0.7±0.2	p = 0.06
Baseline (%)	7.5±1.8	7.8±1.9	7.3±1.8	p = 0.22
Finish line (%)	5.5±1.2	5.7±1.2	5.2±1.3	p = 0.20
Eosinophil				
Baseline	0.2±0.1	0.2±0.1	0.1±0.1	p = 0.14
Finish line	0.0±0.0	0.0±0.1	0.0±0.0	p = 0.72
Baseline (%)	2.8±1.9	3.0±2.1	2.5±1.7	p = 0.22
Finish line (%)	0.3±0.5	0.4±0.7	0.3±0.3	p = 0.78
Basophils				
Baseline	0.1±0.0	0.1±0.0	0.1±0.0	p = 0.95
Finish line	0.1±0.0	0.1±0.0	0.1±0.0	p = 0.89
Baseline (%)	1.2±0.5	1.2±0.5	1.2±0.5	p = 0.85
Finish line (%)	0.8±0.4	0.8±0.4	0.9±0.4	p = 0.60

^a differed significantly from ^b (p < 0.05)

% refer to relative counts; while other values refer to absolute counts

N = total sample; N_m = total male sample; N_f = total female sample; N_c = total caffeine; N_{cm} = total caffeine male; N_{cf} = total caffeine female; N_p = total placebo; N_{pm} = total placebo male; N_{pf} = total placebo female

4.3.2.1 Total white blood cell count

Total white blood cell count levels measured in the caffeine group increased significantly from baseline to the finish line in all subjects (p = 0.00*), and the male (p = 0.00*) and female

($p = 0.00^*$) groups. A similar increase was seen in the placebo group in all subjects ($p = 0.00^*$), as well as the male ($p = 0.00^*$) and female ($p = 0.00^*$) groups. Higher total white blood cell count values were observed at the finish line in the caffeine group compared to placebo ($p = 0.05^*$) (Figure 4.11), as well as in the male group when compared to the female group ($p = 0.01^*$) (Figure 4.12).

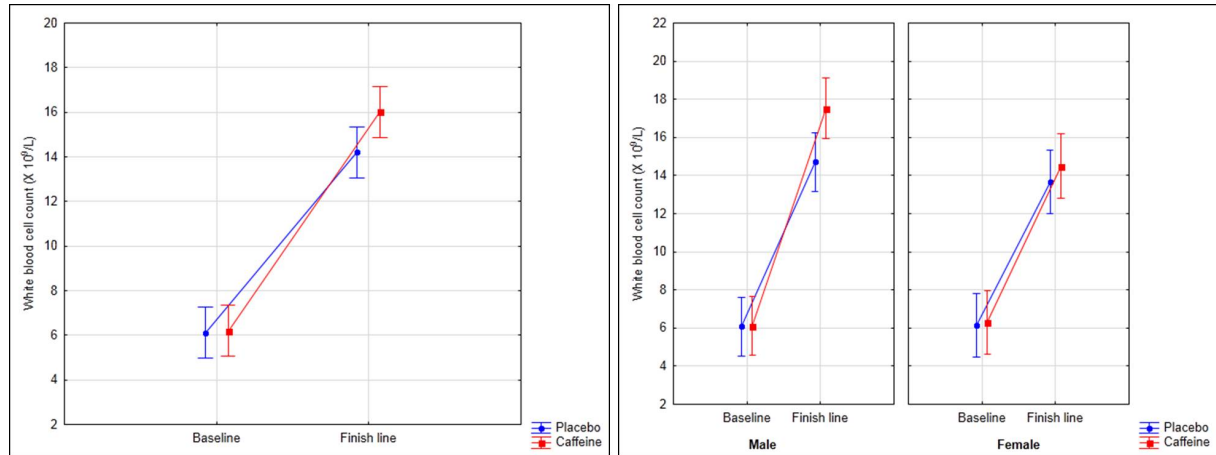


Figure 4.11 Total white blood cell count measured at baseline and at the finish line in the caffeine and placebo groups (Supplementation effect: $p = 0.05^*$) and according to gender (Gender effect: $p = 0.22$)

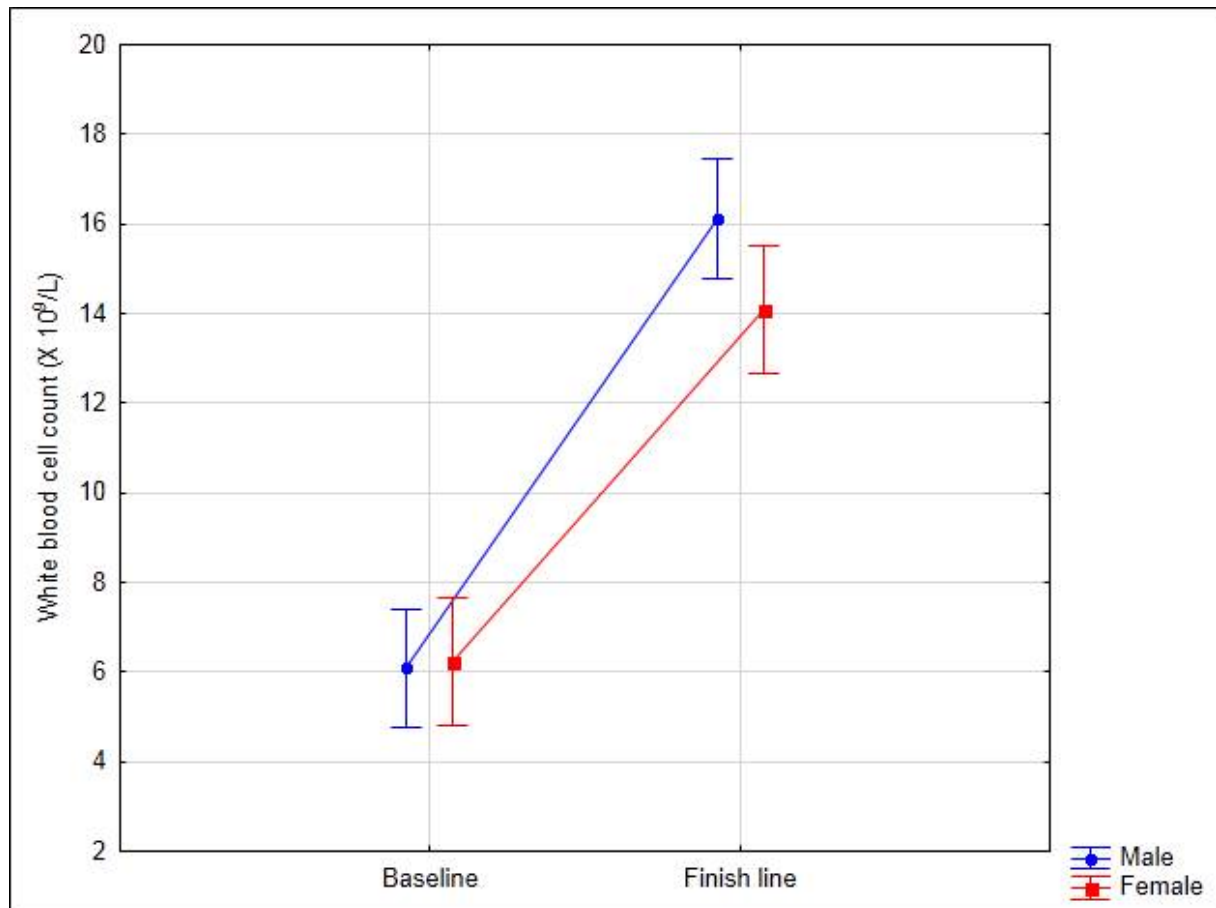


Figure 4.12 Total white blood cell count measured at baseline and at the finish line in males and females (Gender effect: $p = 0.01^*$)

4.3.2.2 Differential white blood cell count

Absolute and relative (percentage) neutrophil counts measured in the caffeine, as well as in the placebo groups increased significantly from baseline to the finish line in all subjects ($p = 0.00^*$), and the male ($p = 0.00^*$) and female ($p = 0.00^*$) groups. Therefore, caffeine supplementation did not influence the absolute ($p = 0.12$) or relative count ($p = 0.97$). Higher neutrophil count values ($p = 0.02^*$) were observed at the finish line in the male group compared to the female group. Lower relative baseline and finish line counts were observed in the male group compared to the female group ($p = 0.00^*$) (Figure 4.13).

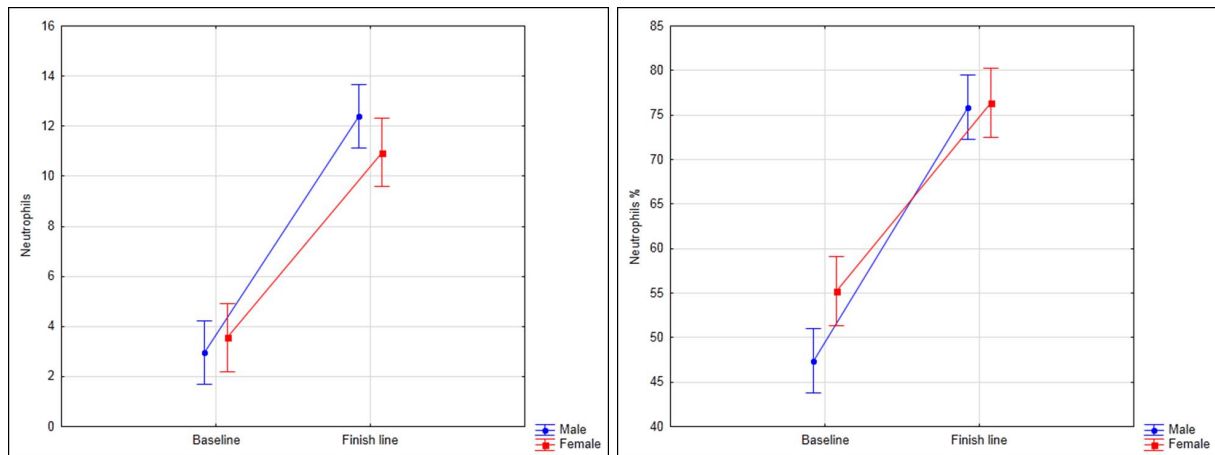


Figure 4.13 Absolute ($p = 0.02^*$) and relative ($p = 0.00^*$) neutrophil count at baseline and at the finish line in males and females (Gender effect: $p < 0.05$)

Absolute lymphocyte count measured in the caffeine group increased significantly from baseline to the finish line all subjects ($p = 0.00^*$) and in the male ($p = 0.00^*$) group, but not in the female ($p = 0.11$) group. The absolute lymphocyte count measured in the placebo group did not differ significantly at baseline and at the finish line in all subjects ($p = 0.31$), and the male ($p = 0.88$) and female ($p = 0.21$) groups.

The increase in the absolute lymphocyte count measured between baseline and at the finish line in all subjects and the male groups was due to caffeine supplementation ($p = 0.05^*$) (Figure 4.14). There was no significant difference in the absolute lymphocyte count measured at baseline and at the finish line between males and females ($p = 0.63$).

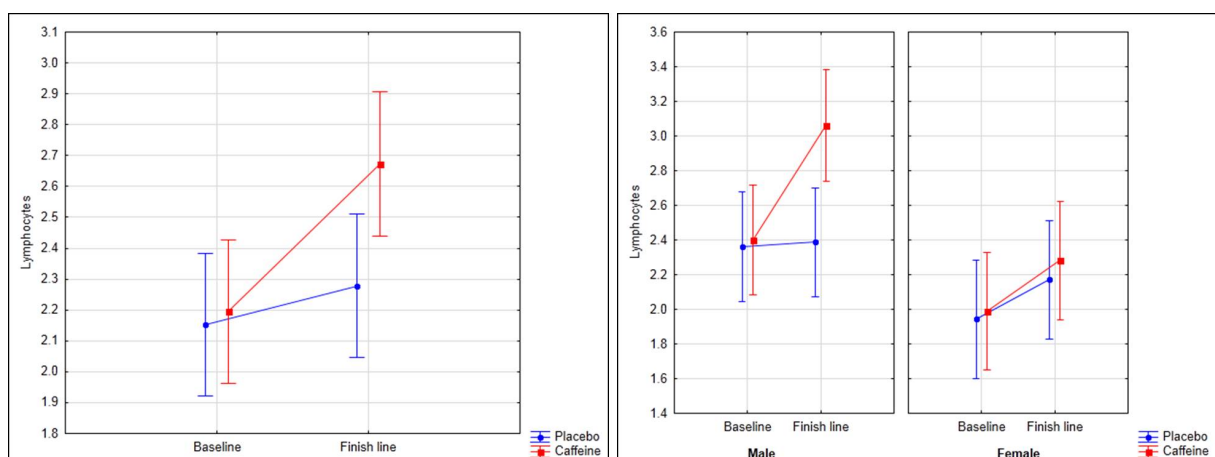


Figure 4.14 The absolute lymphocyte count at baseline and at the finish line in the caffeine and placebo groups (Supplementation effect: $p = 0.05^*$) and according to gender (Gender effect: $p = 0.10$)

The relative lymphocyte count in the caffeine and placebo groups decreased significantly from baseline to the finish line in all subjects ($p = 0.00^*$), and the male ($p = 0.00^*$) and female ($p = 0.00^*$) groups. This decrease was therefore not due to caffeine supplementation ($p = 0.92$).

There was a significant difference in the relative lymphocyte count at baseline and at the finish line between males and females ($p = 0.01^*$). The count observed for males decreased more markedly from baseline to finish line, when compared to the female group (Figure 4.15).

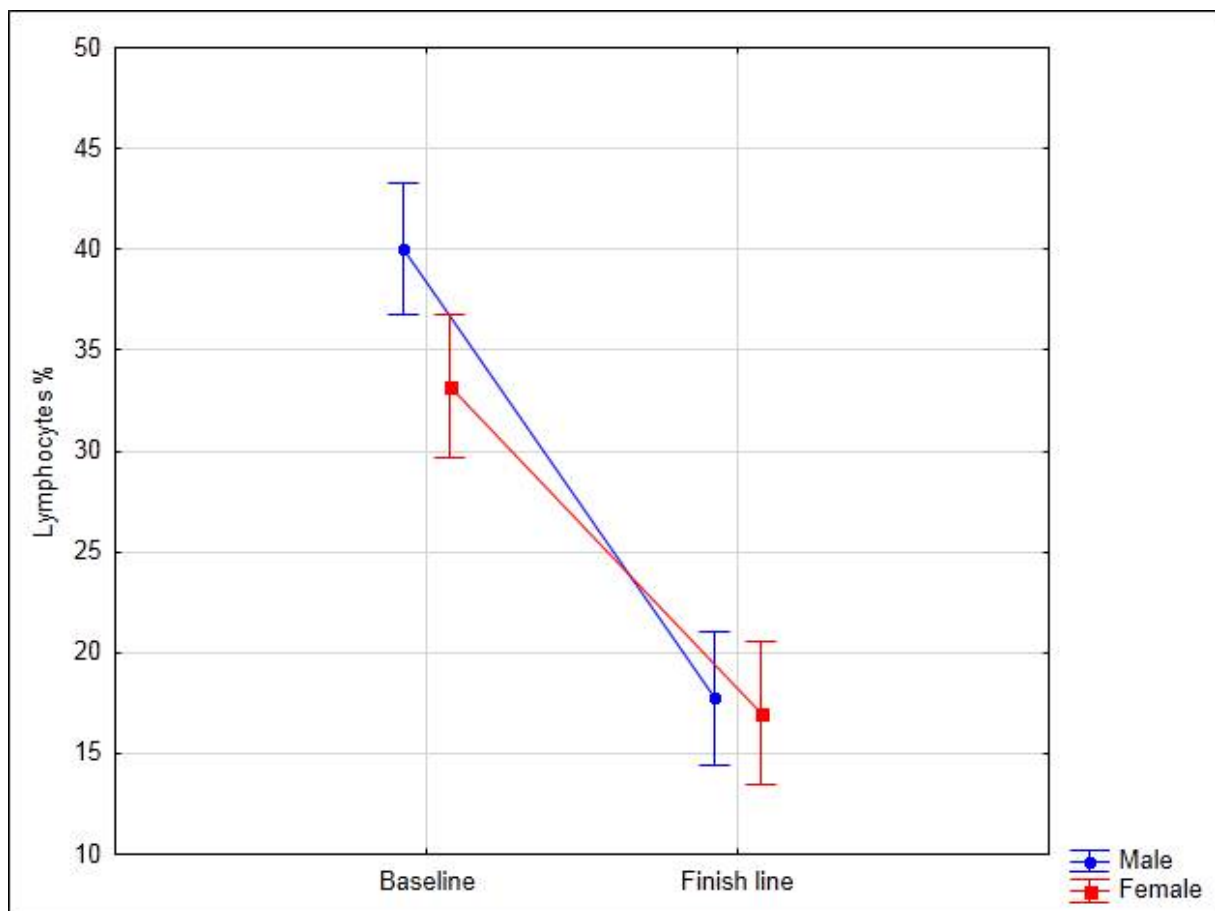


Figure 4.15 The relative lymphocyte percentage at baseline and at the finish line in males and females (Gender effect: $p = 0.01^*$)

The absolute monocyte count measured in the caffeine and placebo groups increased significantly from baseline to the finish line in all subjects ($p = 0.00^*$), and the male ($p = 0.00^*$) and female ($p = 0.00^*$) groups. In contrast, the relative monocyte count measured in the caffeine and placebo groups decreased significantly from baseline to the finish line in all subjects ($p = 0.00^*$), and the male ($p = 0.00^*$) and female ($p = 0.00^*$) groups. These

differences were not due to caffeine supplementation ($p = 0.12$ for absolute and $p = 0.99$ for relative monocyte count).

There was no significant difference between the absolute monocyte count at baseline and at the finish line between males and females ($p = 0.41$). There was, however, a significant difference in the relative monocyte count at baseline and at the finish line between males and females ($p = 0.01^*$) (Figure 4.16).

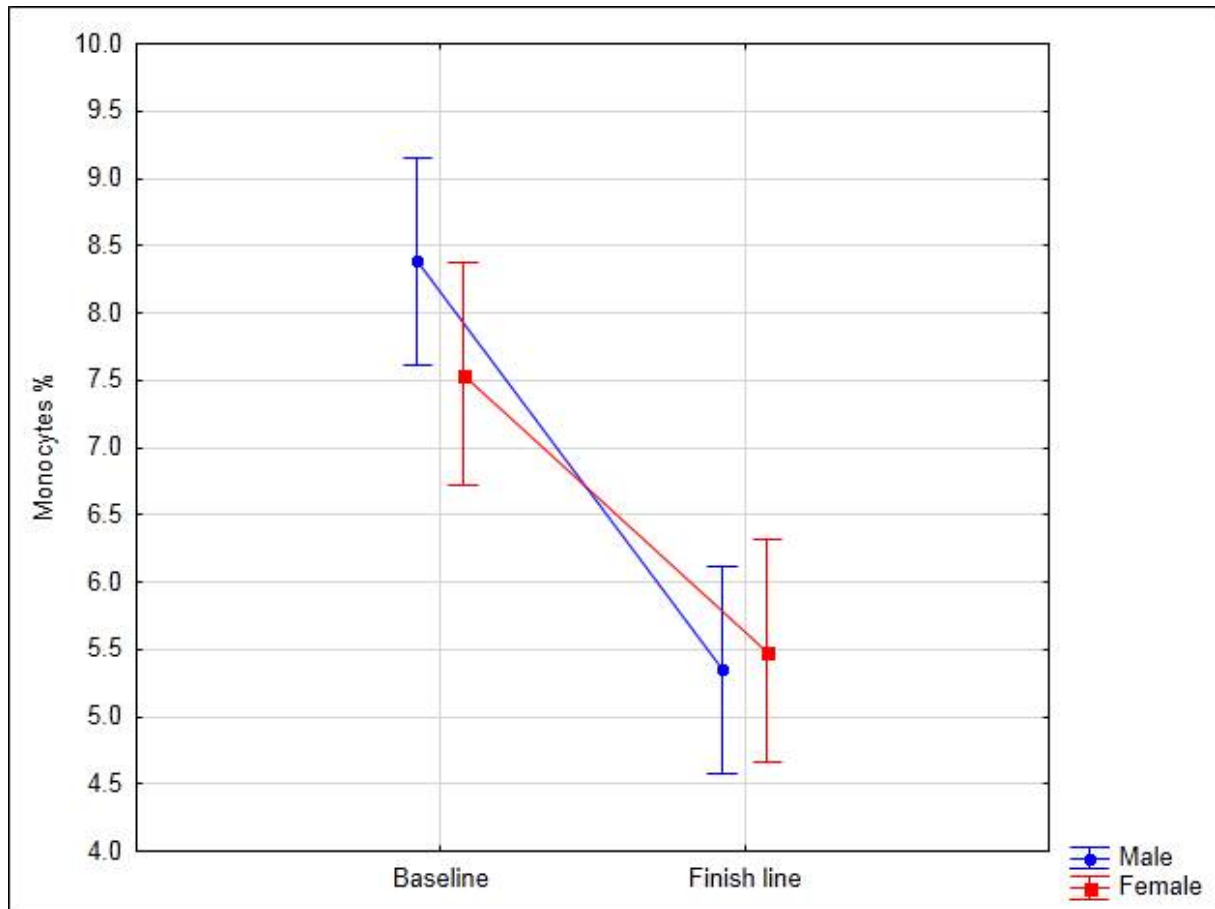


Figure 4.16 The relative monocyte count at baseline and at the finish line in males and females (Gender effect: $p = 0.01^*$)

The absolute and relative eosinophil count in the caffeine and placebo groups decreased significantly from baseline to the finish line in all subjects ($p = 0.00^*$), and the male ($p = 0.00^*$) and female ($p = 0.00^*$) groups. There was no difference in the absolute ($p = 0.77$) or relative ($p = 0.74$) eosinophil count at baseline and at the finish line between the caffeine and placebo groups. There was also no significant difference in the absolute ($p = 0.15$) or relative ($p = 0.28$) eosinophil count measured at baseline and at the finish line between males and females.

The absolute basophil count in the caffeine and placebo group increased significantly from baseline to the finish line in all subjects ($p = 0.00^*$), and the male ($p = 0.00^*$) and female ($p = 0.00^*$) groups. In contrast to this, the relative basophil count in the caffeine and placebo groups, significantly decreased from baseline to finish line in all subjects ($p = 0.00^*$), and the male ($p = 0.00^*$) and female ($p = 0.00^*$) groups. There was no difference in the absolute ($p = 0.38$) or relative ($p = 0.94$) basophil count measured at baseline and at the finish line between the caffeine and placebo groups ($p = 0.38$). Furthermore, the absolute ($p = 0.19$) and relative ($p = 0.83$) basophil count did not differ significantly between males and females.

4.3.3 Plasma lactate

Lactate levels were measured at baseline, during transition (cycle → run) and 3, 6, 9, 12 and 15 minutes after the end of the triathlon (Table 4.7).

Table 4.7 Lactate levels in the caffeine and placebo groups at baseline, during transition (cycle → run) and 3, 6, 9, 12 and 15 minutes after the finish line

	All		Caffeine		Placebo		Caffeine vs. Placebo
	N	Mean±SD	N	Mean±SD	N	Mean±SD	p-value
All	N = 52		N_c = 28		N_p = 28		
Baseline (mmol/l)	41	2.2±0.7	22	1.9±0.5	19	2.6±0.6	p = 0.13
Transition (cycle → run)	46	5.0±2.2	23	5.6±2.4 ^a	23	4.4±1.9 ^b	p = 0.03*
FL 3min (mmol/l)	38	5.3±2.4	18	5.9±2.3	20	4.7±2.5	p = 0.07
FL 6min (mmol/l)	40	4.8±1.6	20	4.7±1.6	20	4.9±1.7	p = 0.76
FL 9min (mmol/l)	45	4.6±1.9	25	5.0±1.4 ^a	20	4.1±2.3 ^b	p = 0.04*
FL 12min (mmol/l)	29	4.4±2.1	15	5.1±2.2 ^a	14	3.7±1.6 ^b	p = 0.02*
FL 15min (mmol/l)	42	4.4±1.8	21	4.7±1.6	21	4.2±1.9	p = 0.45
Male	N_m = 28		N_{cm} = 14		N_{pm} = 14		
Baseline (mmol/l)	23	2.1±0.6	12	1.8±0.5	11	2.5±0.5	p = 0.19
Transition (cycle → run)	26	5.4±2.2	13	6.2±2.3 ^a	13	4.6±1.7 ^b	p = 0.01*
FL 3min (mmol/l)	22	5.5±2.7	10	6.3±1.8 ^a	12	4.8±3.2 ^b	p = 0.03*
FL 6min (mmol/l)	21	4.6±1.9	10	4.5±1.6	11	4.7±2.2	p = 0.95
FL 9min (mmol/l)	24	4.5±2.2	13	5.0±1.4	11	4.0±2.8	p = 0.14
FL 12min (mmol/l)	14	4.5±2.1	8	5.1±2.6	6	3.6±1.0	p = 0.10
FL 15min (mmol/l)	20	4.5±1.9	10	4.8±1.5	10	4.3±2.3	p = 0.73
Female	N_f = 24		N_{cf} = 12		N_{pf} = 12		
Baseline (mmol/l)	18	2.4±0.7	10	2.0±0.6	8	2.8±0.7	p = 0.40
Transition (cycle → run)	20	4.6±2.1	10	4.8±2.2	10	4.3±2.1	p = 0.48
FL 3min (mmol/l)	16	5.0±2.1	8	5.3±2.8	8	4.6±1.0	p = 0.61
FL 6min (mmol/l)	19	5.0±1.4	10	4.9±1.6	9	5.1±1.2	p = 0.71
FL 9min (mmol/l)	21	4.7±1.6	12	5.1±1.4	9	4.2±1.7	p = 0.15
FL 12min (mmol/l)	15	4.4±2.0	7	5.1±2.0	8	3.8±2.0	p = 0.13
FL 15min (mmol/l)	22	4.3±1.7	11	4.6±1.7	11	4.1±1.7	p = 0.47

^a differed significantly from ^b (p < 0.05)

FL: Finish line

N = total sample; N_m = total male sample; N_f = total female sample; N_c = total caffeine; N_{cm} = total caffeine male; N_{cf} = total caffeine female; N_p = total placebo; N_{pm} = total placebo male; N_{pf} = total placebo female

The researcher performed a RMANOVA with VEPAC on lactate levels with factors group, caffeine and stage (baseline, transition (cycle → run) and at 3, 6, 9, 12 and 15 minutes after the finish line) and then gender, caffeine and stage, with respondents nested firstly in group (p = 0.61) and then in gender (p = 0.92).

There was a significant difference in the lactate levels measured at baseline, during the transition (cycle → run) and at 3, 6, 9, 12 and 15 minutes after the finish line between the caffeine and placebo groups (p = 0.04*) (Figure 4.17). There were, however, no significant differences in lactate levels measured at baseline, during transition (cycle → run) and at 3, 6, 9, 12 and 15 minutes after the finish line between males and females (p = 0.56)

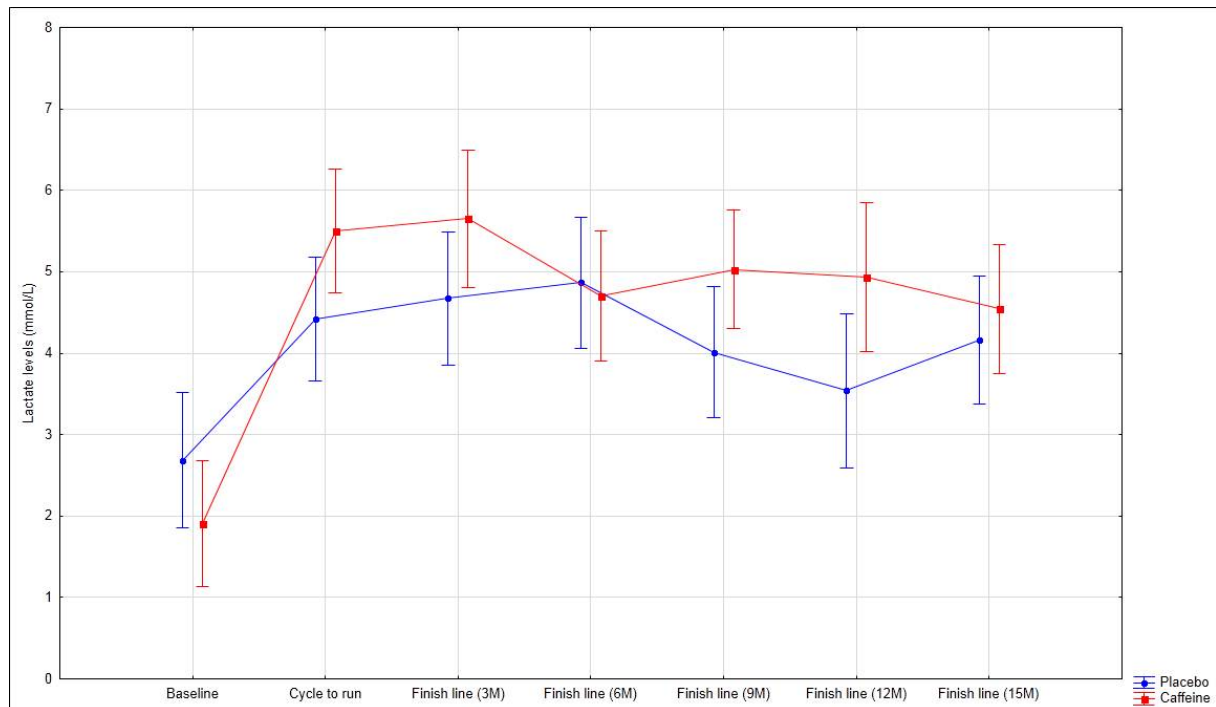


Figure 4.17 Lactate levels measured at baseline, during transition (cycle → run) and at 3, 6, 9, 12 and 15 minutes after the finish line in the caffeine and placebo groups (Supplementation effect: $p = 0.04^*$)

4.3.3.1 Influence of lactate levels on the overall time to complete the triathlon

Basic statistics, by means of correlations dialog, was used to determine which of the lactate measurements at the various time points, influenced the overall time to complete the triathlon. None of the lactate measurements influenced the overall time to complete the triathlon according to the correlations dialog and therefore a factorial ANOVA with VEPAC and lactate levels as covariate was not conducted.

4.4 Factors influencing the ergogenic effect of caffeine supplementation

4.4.1 Habitual caffeine intake

The habitual caffeine intake, shown in Table 4.8 did not influence the baseline caffeine levels before T1 ($p = 0.88$) or T2 ($p = 0.60$), as determined by the non-parametric Spearman R test.

Table 4.8 Habitual caffeine intake

	All (<i>N</i> = 26)	Male (<i>N_m</i> = 14)	Female (<i>N_f</i> = 12)
	Mean±SD	Mean±SD	Mean±SD
Habitual caffeine intake (mg/day)*	412.7±504.8	337.1±345.7	501.0±649.7

N = total sample; *N_m* = total male sample; *N_f* = total female sample

4.4.2 Effect of the pre-event meal on plasma caffeine levels

Details of the pre-event meal of the caffeine and placebo groups are displayed in Table 4.9. There were no statistically significant differences between the caffeine and placebo groups with regard to components of the pre-event meal, except for protein intake measured in g/kg BW in the male group ($p = 0.01^*$). However, when comparing the protein intake measured in g, there were no statistically significant difference between the caffeine and placebo groups ($p = 0.34$) (Table 4.9).

Table 4.9 Pre-event meal of the caffeine and placebo groups

	Recommendation	Caffeine	Placebo	Caffeine vs. Placebo
All (<i>N</i> = 52)		Mean±SD	Mean±SD	p-value
Total energy intake (kcal)	N/A	323.1±239.2	302.6±234.4	$p = 0.76$
Total energy intake (kcal/kg BW)	N/A	4.6±3.0	4.3±3.2	$p = 0.73$
Protein intake (g)	N/A	9.2±8.5	7.5±6.7	$p = 0.43$
Protein intake (g/kg BW)	0.15-0.25 g/kg BW*	0.1±0.1	0.1±0.1	$p = 1.00$
CHO intake (g)		48.0±32.3	46.4±42.0	$p = 0.88$
CHO intake (g/kg BW)	1-4 g/kg BW (Pre-event fuelling, exercise > 60 minutes)	0.7±0.4	0.7±0.5	$p = 1.00$
Fat intake (g)	N/A	9.1±10.8	8.3±11.1	$p = 0.79$
Fat intake (g/kg BW)	N/A	0.1±0.1	0.1±0.2	$p = 1.00$
Male (<i>N_m</i> = 28)		Mean±SD	Mean±SD	p-value
Total energy intake (kcal)	N/A	429.5±263.3	375.7±254.5	$p = 0.59$
Total energy intake (kcal/kg BW)	N/A	5.6±3.3	5.0±3.2	$p = 0.63$
Protein intake (g)		12.2±10.3	8.8±8.2	$p = 0.34$
Protein intake (g/kg BW)	0.15-0.25 g/kg BW*	0.2±0.1 ^a	0.1±0.1 ^b	$p = 0.01^*$
CHO intake (g)		62.6±33.4	59.4±49.4	$p = 0.84$
CHO intake (g/kg BW)	1-4 g/kg BW	0.8±0.4	0.8±0.6	$p = 1.00$
Fat intake (g)	N/A	12.7±13.4	9.4±13.3	$p = 0.52$
Fat intake (g/kg BW)	N/A	0.2±0.2	0.1±0.2	$p = 0.20$
Female (<i>N_f</i> = 24)		Mean±SD	Mean±SD	p-value
Total energy intake (kcal)	N/A	207.0±145.5	216.2±188.0	$p = 0.89$
Total energy intake (kcal/kg BW)	N/A	3.4±2.4	3.5±3.1	$p = 0.11$
Protein intake (g)		5.9±4.5	5.8±4.0	$p = 0.95$
Protein intake (g/kg BW)	0.15-0.25 g/kg BW*	0.1±0.1	0.1±0.1	$p = 1.00$
Carbohydrate intake (g)		32.0±23.2	31.1±25.4	$p = 0.93$
CHO intake (g/kg BW)	1-4 g/kg BW	0.5±0.4	0.5±1.6	$p = 1.00$
Fat intake (g)	N/A	5.1±5.0	6.9±8.0	$p = 0.52$
Fat intake (g/kg BW)	N/A	0.1±0.1	0.1±0.1	$p = 1.00$

Sources: (4, 169, 170, 172, 174, 175, 183, 203)

^a differed significantly from ^b ($p < 0.05$)

kcal: calories; kcal/kg BW/day: calories per kilogram body weight per day; kcal/kg FFM: calories per kilogram fat free mass; g/kg BW: gram per kilogram body weight; N/A: not applicable; N = total sample; *N_m* = total male sample; *N_f* = total female sample

There were no statistically significant correlations between any of the listed dietary components of the pre-event meal and plasma caffeine levels in the group receiving caffeine supplementation, suggesting that the pre-event meal did not influence the absorption of caffeine after supplementation.

4.4.3 Menstrual history (females)

4.4.3.1 Menstrual patterns and oral contraceptive use

Currently only 50% ($N = 6$) of the females in the sample group have regular menstrual cycles. Forty two percent of the females ($N = 5$) were post-menopausal. Of all the females, 42% ($N = 5$) had gone more than three months without menstruation during the previous 12 months, all of which reported loss of menstruation due to menopause. Fifty eight percent ($N = 7$) of the females used oral contraceptive medication (Table 4.10).

Table 4.10 Menstrual patterns and oral contraceptive use

	% ($N_f = 12$)
General information	
Age of menarche (mean \pm SD)	12.9 \pm 1.8
Menarche reached before onset of sport	75% ($N = 9$)
Gynecological age (mean \pm SD)	24.3 \pm 12.1
Menstrual cycle information	
Eumenorrhea	50% ($N = 6$)
Oligomenorrhea	8% ($N = 1$)
Amenorrhea	0% ($N = 0$)
No cycles preceding 12 months	42% ($N = 5$)
No cycle due to menopause	42% ($N = 5$)
Changes in response to training (can be more than one)	
Shorter cycle	17% ($N = 2$)
Longer cycle	8% ($N = 1$)
Lighter bleeding	17% ($N = 2$)
No cycle > 3 months	17% ($N = 2$)
Contraceptive use	
Yes	58% ($N = 7$)
No	42% ($N = 5$)
Self-reported menstrual phase during triathlon 1	
Luteal phase	50% ($N = 6$)
Follicular phase	8% ($N = 1$)
Self-reported menstrual phase during triathlon 2	
Luteal phase	8% ($N = 1$)
Follicular phase	50% ($N = 6$)

N_f = total female sample

4.4.3.2 The influence of the phase of menstrual cycle on plasma caffeine levels during transition (cycle → run) and at the finish line in the caffeine and placebo groups

This analysis could not be performed as there were no/limited observations, when the small female subject sample was further sub-divided into the different phases of the menstrual cycle.

4.4.3.3 The influence of oral contraceptive use on plasma caffeine levels during transition (cycle → run) and at the finish line in the caffeine and placebo groups

The researcher did a factorial ANOVA with variance, estimation, precision and comparison (VEPAC) of plasma caffeine levels during transition (cycle → run) and at the finish line with factors group (whether or not subjects received caffeine supplementation during T1 or T2) and caffeine, with respondents nested in group. To investigate the influence of oral contraceptive use on plasma caffeine levels during transition (cycle → run) and at the finish line respectively, these variables were one by one entered as covariates in a similar analysis of covariance with factors (group and caffeine) as described above.

The introduction of oral contraceptive use as a covariate had no significant effect on plasma caffeine levels measured during transition (cycle → run) ($p = 0.63$ with group) or the plasma caffeine levels measured at the finish line ($p = 0.06$ with group) (Figure 4.18).

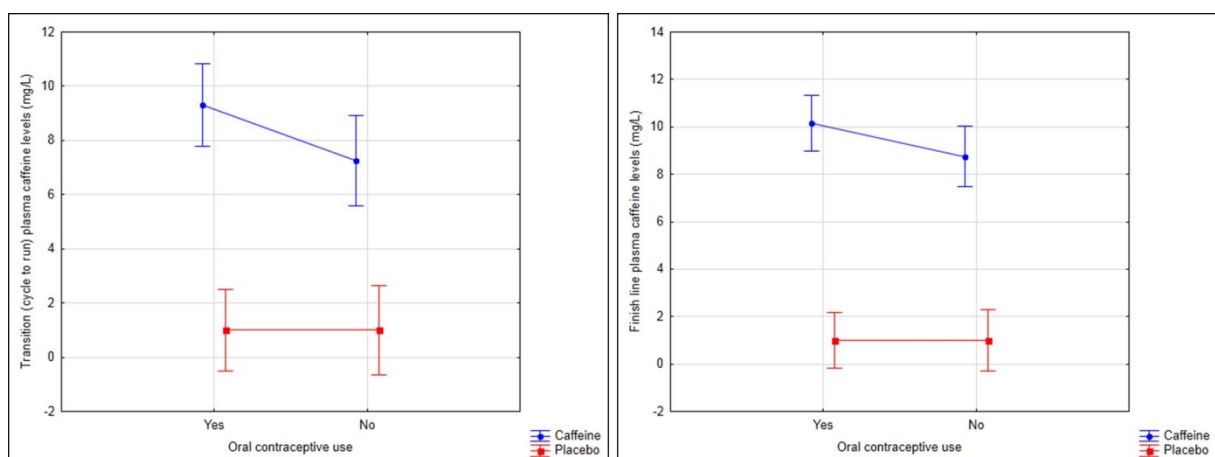


Figure 4.18 Plasma caffeine levels measured during transition (cycle → run) ($p = 0.63$) and at the finish line ($p = 0.06$) according to oral contraceptive use and caffeine supplementation.

4.4.3.4 Descriptive information on the phase of menstrual cycle and oral contraceptive use on the overall time to complete the triathlon in the caffeine and placebo groups

The researcher did a factorial ANOVA with VEPAC of the overall time to complete the triathlon with factors group (whether or not subjects received caffeine supplementation during T1 or T2) and caffeine, with respondents nested in group. To investigate the influence of the phase of the menstrual cycle and oral contraceptive use respectively, on the overall time to complete the triathlon, these variables were one by one entered as covariates in similar analysis of covariance with factors (group and caffeine) as described above.

The introduction of the self-reported phase of the menstrual cycle as a covariate had no significant effect on the overall time to complete the triathlon ($p = 0.29$ with group). The introduction of oral contraceptive use as a covariate also had no significant effect on the overall time to complete the triathlon ($p = 0.73$ with group).

As mentioned above, the self-reported phase of the menstrual cycle did not significantly affect the overall time to complete the triathlons, irrespective of whether subjects were supplemented with caffeine or placebo, and irrespective of oral contraceptive use (Table 4.11, Figure 4.19). Although not statistically significant, it may be of clinical importance that time to completion was slightly faster (4%) in the follicular phase when compared to the luteal phase, in the caffeine group only. For both the caffeine and placebo groups, average overall time to complete the triathlon was increased in post-menopausal women when compared to pre-menopausal women. The average overall time to complete the triathlon was ≈ 25 minutes (14%) slower for the post-menopausal females, when compared to the females in the luteal phase of the menstrual cycle, independent of supplementation, while it was 28.83 (± 14.16) minutes and 21.77 (± 8.28) minutes slower for the caffeine and placebo post-menopausal groups respectively, when compared to the females in the follicular phase of the menstrual cycle. Although this finding was not statistically significant, in our opinion these differences of 16% and 13% respectively are indeed of clinical significance.

Table 4.11 Overall time to complete the triathlons (minutes) according to the phase of the menstrual cycle and oral contraceptive use

	Caffeine ($N_{cf} = 12$)		Placebo ($N_{pf} = 12$)		Caffeine vs. Placebo
	Mean (\pm SE)	N	Mean (\pm SE)	N	p-value
All ($N_f = 24$)	155.75 (± 6.23)	12	157.39 (± 6.23)	12	p = 0.48
Luteal phase	149.08 (± 4.44)	3	148.15 (± 4.40)	4	p = 0.96
Post-menopausal	173.49 (± 14.48)	5	172.89 (± 12.85)	5	p = 0.84
Follicular phase	144.66 (± 2.16)	4	151.12 (± 4.57)	3	p = 0.76
Oral contraceptive (Yes)	157.07 (± 9.17)	7	156.73 (± 9.14)	7	p = 0.95
Oral contraceptive (No)	158.78 (± 10.81)	5	162.65 (± 10.81)	5	p = 0.57

N_f = total female sample; N_{cf} = total caffeine female; N_{pf} = total placebo female

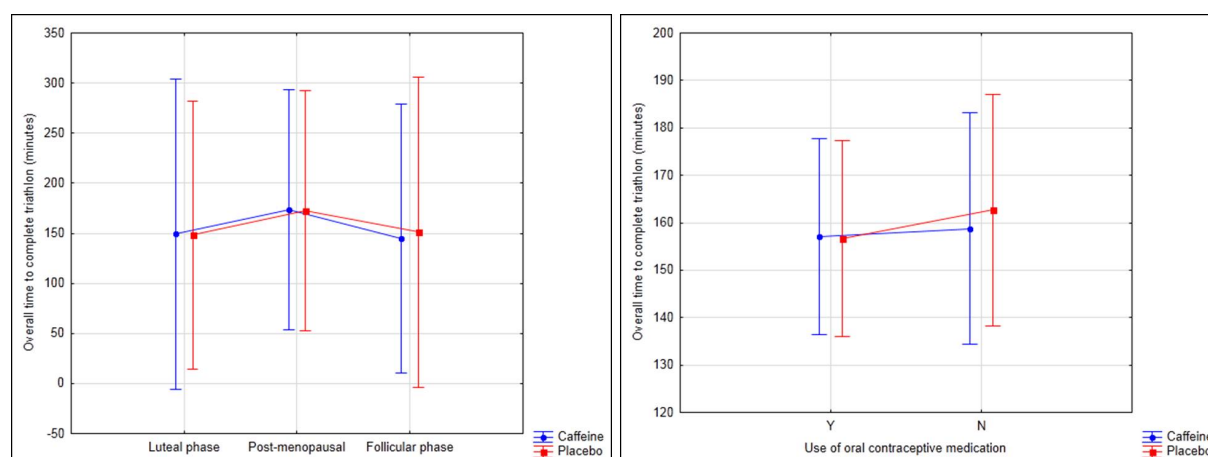


Figure 4.19 The effect of the phase of the menstrual cycle ($p = 0.66$) and oral contraceptive use ($p = 0.16$) on the overall time to complete the triathlon (minutes) in the caffeine and placebo groups.

4.4.4 Genetic analysis

4.4.4.1 *CYP1A2* genotype and allele frequencies

The *CYP1A2**1F allele (rs762551-C) is common with highly comparable frequencies in various populations (160). The frequency of the variant C allele was 0.29 in the study population in the present study. Caffeine metabolism is the same for heterozygotes and CC homozygotes. Triathletes were therefore grouped as AA homozygous (62%, $N = 16$) and C-allele carriers (38%, $N = 10$); the latter group including both heterozygotes and CC homozygotes (Table 4.12) as done in previous studies (158, 165, 208).

The allelic frequency of the *CYP1A2**1D (rs35694136delT) allele in the 5'-flanking region (160) is 0.08, with 15% ($N = 4$) of the study group being heterozygous for this genotype (Table 4.12).

The frequency of the T-allele of the SNP located at 15q24 between *CYP1A1* and *CYP1A2* (rs2472297-T) (163) is 0.27 in the study population, with 23% ($N = 6$) being heterozygous, and 15% ($N = 4$) being homozygous for the T/T genotype (Table 4.12).

The frequency of the A allele of the SNP mapped to 15q24 within the bidirectional promoter of the *CYP1A1-CYP1A2* locus (164) (rs2470893-A) was 0.31, with 31% ($N = 8$) being heterozygous, and 15% ($N = 4$) being homozygous for the A/A genotype (Table 4.12).

Variant rs6968865-T is located within a linkage disequilibrium (LD) block of 240kb on 7p15. The gene encoding the aryl hydrocarbon receptor (*AHR*) is the only gene located within this LD block. *AHR* plays a central role in xenobiotic metabolism and induces members of the *CYP1* family of genes (*CYP1A1*, *CYP1A2* and *CYP1B1*) (163). The frequency of the T allele in the study population was 0.60, with 58% ($N = 15$) being heterozygous and 31% ($N = 8$) being homozygous (Table 4.12).

Table 4.12 Genotype and allele frequencies:

	All ($N=26$)		Male ($N_m=14$)		Female ($N_f=12$)	
	Genotype frequency % (N)	Allele frequency	Genotype frequency % (N)	Allele frequency	Genotype frequency % (N)	Allele frequency
<i>CYP1A2*1F (rs762551-C)</i>						
C/C	19% ($N = 5$)	C = 0.29	21% ($N = 3$)	C = 0.29	17% ($N = 2$)	C = 0.29
A/C	19% ($N = 5$)		14% ($N = 2$)		25% ($N = 3$)	
A/A	62% ($N = 16$)	A = 0.71	65% ($N = 9$)	A = 0.71	58% ($N = 7$)	A = 0.71
<i>CYP1A2*1D (rs35694136delT)</i>						
T/T	85% ($N = 22$)	T = 0.92	86% ($N = 12$)	T = 0.93	83% ($N = 10$)	T = 0.92
-/T	15% ($N = 4$)		14% ($N = 2$)		17% ($N = 2$)	
-/-	0%	- = 0.08	0%	- = 0.07	0%	- = 0.08
Between <i>CYP1A1-CYP1A2 (rs2472297-T)</i>						
C/C	62% ($N = 16$)	C = 0.73	72% ($N = 10$)	C = 0.79	50% ($N = 6$)	C = 0.67
C/T	23% ($N = 6$)		14% ($N = 2$)		33% ($N = 4$)	
T/T	15% ($N = 4$)	T = 0.27	14% ($N = 2$)	T = 0.21	17% ($N = 2$)	T = 0.33
Between <i>CYP1A1-CYP1A2 (rs2470893-A)</i>						
G/G	54% ($N = 14$)	G = 0.69	65% ($N = 9$)	G = 0.75	41.5% ($N = 5$)	G = 0.63
G/A	31% ($N = 8$)		21% ($N = 3$)		41.5% ($N = 5$)	
A/A	15% ($N = 4$)	A = 0.31	14% ($N = 2$)	A = 0.25	17% ($N = 2$)	A = 0.37
Near <i>AHR</i> gene (rs698865-T)						
T/T	31% ($N = 8$)	T = 0.60	36% ($N = 5$)	T = 0.64	25% ($N = 3$)	T = 0.54
A/T	58% ($N = 15$)		57% ($N = 8$)		58% ($N = 7$)	
A/A	12% ($N = 3$)	A = 0.40	7% ($N = 1$)	A = 0.36	17% ($N = 2$)	A = 0.46

N = total sample; N_m = total male sample; N_f = total female sample

4.4.4.2 Influence of genotype on plasma caffeine levels

The researcher did a RMANOVA with VEPAC on plasma caffeine levels with factors group (whether subjects received caffeine supplementation during T1 or T2), caffeine supplementation and genotype frequency and then gender, caffeine supplementation and genotype frequency with respondents nested firstly in group and i) *CYP1A2*1F* (rs762551-C) ($p = 0.08$), ii) *CYP1A2*1D* (rs35694136delT) ($p = 0.54$), iii) *CYP1A1-CYP1A2* (rs2472297-T) ($p = 0.46$), iv) *CYP1A1-CYP1A2* (rs2470893-A) ($p = 0.11$), iv) the variant near the *AHR* gene (rs 6968865-T) ($p = 0.10$) and then in gender and i) *CYP1A2*1F* (rs762551-C) ($p = 0.51$), ii) *CYP1A2*1D* (rs35694136delT) ($p = 0.33$), iii) *CYP1A1-CYP1A2* (rs2472297-T) ($p = 0.78$), iv) *CYP1A1-CYP1A2* (rs2470893-A) ($p = 0.91$) and v) the variant near the *AHR* gene (rs 6968865-T) ($p = 0.12$). Therefore, no carry-over effects from whether or not the subject received caffeine during T1 or T2 was observed when analysing the influence of the genotype frequency on plasma caffeine levels.

There were no significant interaction between plasma caffeine levels measured at baseline, during transition (cycle → run) and at the finish line and the genotype frequency of i) *CYP1A2*1F* (rs762551-C) ($p = 0.61$), ii) *CYP1A2*1D* (rs35694136delT) ($p = 0.88$), iii) *CYP1A1-CYP1A2* (rs2472297-T) ($p = 0.27$), iv) *CYP1A1-CYP1A2* (rs2470893-A) ($p = 0.21$) or v) the variant near the *AHR* gene (rs 6968865-T) ($p = 0.39$) in the caffeine and placebo groups.

There were no significant interaction between plasma caffeine levels measured at baseline, during transition (cycle → run) and at the finish line and the genotype frequency of i) *CYP1A2*1F* (rs762551-C) ($p = 0.69$), ii) *CYP1A2*1D* (rs35694136delT) ($p = 0.51$), iii) *CYP1A1-CYP1A2* (rs2472297-T) ($p = 0.78$), iv) *CYP1A1-CYP1A2* (rs2470893-A) ($p = 0.91$) or v) the variant near the *AHR* gene (rs 6968865-T) ($p = 0.12$) in the male or female group.

4.4.4.3 Influence of genotype frequency on the overall time to complete the triathlon

The researcher did a factorial ANOVA with VEPAC on the overall time to complete the triathlon with factors group and caffeine and then gender and caffeine with respondents nested firstly in group and then nested in gender.

The introduction of i) *CYP1A2*1F* (rs762551-C) ($p = 0.71$ with group and $p = 0.26$ with gender), ii) *CYP1A2*1D* (rs35694136delT) ($p = 0.99$ with group and $p = 0.96$ with gender), iii) *CYP1A1-CYP1A2* (rs2472297-T) ($p = 0.85$ with group)), iv) *CYP1A1-CYP1A2*

(rs2470893-A) ($p = 0.32$ with group and $p = 0.44$ with gender) and v) the variant near the *AHR* gene (rs 6968865-T) ($p = 0.63$ with group) had no significant effect on the overall time to complete the triathlon. Due to the limited number of observations when divided into gender, the effect of covariates *CYP1A1-CYP1A2* (rs2472297-T) and the variant near the *AHR* gene (rs 6968865-T) on overall time to complete the triathlon according to gender could not be established.

4.5 Factors influencing triathlon performance

4.5.1 Medical history and over-the-counter supplement use

The subjects were, overall, in excellent health with few chronic diseases evident (Table 4.9). Supplement use was rife among the subjects, with 85% ($N = 22$) using supplements. All the females reported using supplements (100%, $N_f = 12$), while this was the case with only 75% ($N_m = 10$) of the males. The supplements most commonly used were multivitamin and mineral supplements (85%, $N = 22$), as well as energy drinks and bars (85%, $N = 22$) (Table 4.13).

Table 4.13 Medical history and supplement use

	All (N =26)		Male (N_m=14)		Female (N_f=12)	
Medical history	%	N	%	N _m	%	N _f
Injury or illness	12%	3	21%	3	0%	0
History of stress fractures	0%	0	0%	0	0%	0
Chronic illness ^a	12%	3	14%	2	8%	1
Allergies ^b	19%	5	14%	2	25%	3
OTC medication ^c	35%	9	21%	3	50%	6
Prescribed medication ^d	23%	6	14%	2	33%	4
Inhaler	8%	2	0%	0	17%	2
High blood pressure	0%	0	0%	0	0%	0
Heart disease	0%	0	0%	0	0%	0
High total cholesterol	8%	2	14%	2	0%	0
Diabetes mellitus	0%	0	0%	0	0%	0
	%	N	%	N _m	%	N _f
Supplement use	85%	22	71%	10	100%	12
Multivitamin & mineral	85%	22	79%	11	92%	11
Individual vitamins	50%	13	50%	7	50%	6
Individual minerals	27%	7	21%	3	33%	4
Protein supplements	42%	11	29%	4	58%	7
Herbal supplements ^e	12%	3	14%	2	8%	1
Energy drinks & bars	85%	22	71%	10	100%	12
Creatine	12%	3	14%	2	8%	1
Single amino acids	19%	5	14%	2	25%	3
Frequency	%	N	%	N _m	%	N _f
Daily	31%	8	29%	4	33%	4
Specific times	35%	9	14%	2	58%	7
Occasionally	31%	8	29%	4	33%	4
Several times a week	8%	2	7%	1	8%	1
Reasons	%	N	%	N _m	%	N _f
Inadequate diet	35%	9	29%	4	42%	5
Treat illness / injury	8%	2	7%	1	8%	1
Increase muscle mass	8%	2	7%	1	8%	1
Prevent illness	46%	12	57%	8	33%	4
Weight loss	0%	0	0%	0	0%	0
Increased energy	35%	9	21%	3	50%	6
Enhance exercise performance	31%	8	21%	3	42%	5
No reason	4%	1	0%	0	8%	1

^aChronic illness: any condition requiring long term prescription treatment ; ^bAllergies: food or non-food related; ^cOTC Over the counter medication; ^dPrescribed medication: any medication used on a repeated basis; prescribed by a medical doctor; ^eHerbal supplements: includes all herbal preparations

N = total sample; N_m = total male sample; N_f = total female sample

4.5.2 Full blood count

A full blood count was measured at baseline and again at the finish line (Table 4.14).

Table 4.14 Full blood count values in the caffeine and placebo groups

	All	Caffeine	Placebo	Caffeine vs. Placebo
	Mean±SD	Mean±SD	Mean±SD	p-value
All	N = 52	N_c = 26	N_p = 26	
White blood cells				
Baseline (X 10 ⁹ /l)	6.2±1.6	6.2±1.8	6.1±1.4	p = 0.88
Finish line (X 10 ⁹ /l)	15.2±3.9	16.1±4.2 ^a	14.2±3.5 ^b	p = 0.01*
Red blood cell count				
Baseline (X 10 ¹² /l)	4.6±0.4	4.6±0.4	4.6±0.4	p = 0.57
Finish line (X 10 ¹² /l)	4.5±0.4	4.5±0.4	4.5±0.3	p = 0.30
Hemoglobin				
Baseline (mg %)	14.6±1.2	14.7±1.4	14.6±1.0	p = 0.86
Finish line (mg %)	14.4±1.4	14.3±1.5	14.4±1.2	p = 0.77
Heamatocrit				
Baseline (%)	41.4±2.6	41.5±2.7	41.3±2.7	p = 0.48
Finish line (%)	40.4±2.8	40.2±2.9	40.7±2.8	p = 0.24
Platelets				
Baseline (X 10 ⁹ /l)	295.7±51.7	295.1±51.0	296.3±53.4	p = 0.87
Finish line (X 10 ⁹ /l)	380.1±69.2	383.6±70.9	376.7±68.7	p = 0.44
Male	N_m = 28	N_{cm} = 14	N_{pm} = 14	
White blood cells				
Baseline (X 10 ⁹ /l)	6.1±1.3	6.1±1.4	6.1±1.3	p = 0.85
Finish line (X 10 ⁹ /l)	16.1±4.0	17.6±4.2 ^a	14.7±3.4 ^b	p = 0.00*
Red blood cell count				
Baseline (X 10 ¹² /l)	4.9±0.3	4.9±0.3	4.9±0.3	p = 0.87
Finish line (X 10 ¹² /l)	4.8±0.3	4.8±0.4	4.8±0.3	p = 0.93
Hemoglobin				
Baseline (mg %)	15.3±0.9	15.4±1.1	15.2±0.7	p = 0.60
Finish line (mg %)	15.0±1.1	15.2±1.2	14.9±1.1	p = 0.39
Heamatocrit				
Baseline (%)	43.1±1.7	43.0±1.9	43.3±1.6	p = 0.61
Finish line (%)	42.2±2.0	42.0±2.1	42.5±2.0	p = 0.43
Platelets				
Baseline (X 10 ⁹ /l)	286.3±50.1	285.6±53.4	287.0±48.5	p = 0.89
Finish line (X 10 ⁹ /l)	369.4±63.9	375.0±69.2	364.2±60.7	p = 0.34
Female	N_f = 24	N_{cf} = 12	N_{pf} = 12	
White blood cells				
Baseline (X 10 ⁹ /l)	6.2±1.9	6.3±2.3	6.1±1.6	p = 0.86
Finish line (X 10 ⁹ /l)	14.1±3.6	14.5±3.8	13.7±3.6	p = 0.35
Red blood cell count				
Baseline (X 10 ¹² /l)	4.4±0.3	4.4±0.3	4.3±0.2	p = 0.35
Finish line (X 10 ¹² /l)	4.2±0.3	4.2±0.3	4.3±0.2	p = 0.17
Hemoglobin				
Baseline (mg %)	13.8±1.0	13.8±1.2	13.9±0.9	p = 0.80
Finish line (mg %)	13.6±1.2	13.3±1.3	13.9±1.1	p = 0.22
Heamatocrit				
Baseline (%)	39.4±2.0	39.7±2.4	39.0±1.6	p = 0.16
Finish line (%)	38.4±2.2	38.2±2.4	38.7±2.0	p = 0.37
Platelets				

	All	Caffeine	Placebo	Caffeine vs. Placebo
	Mean±SD	Mean±SD	Mean±SD	p-value
Baseline (X 10 ⁹ /l)	306.8±52.4	306.3±47.7	307.3±58.8	p = 0.93
Finish line (X 10 ⁹ /l)	392.2±74.1	393.0±74.6	391.3±77.0	p = 0.88

^a differed significantly from ^b (p < 0.05)

% refer to relative counts; while other values refer to absolute counts

N = total sample; N_m = total male sample; N_f = total female sample; N_c = total caffeine; N_{cm} = total caffeine male; N_{cf} = total caffeine female; N_p = total placebo; N_{pm} = total placebo male; N_{pf} = total placebo female

The researcher performed a RMANOVA with VEPAC on total white blood cell count levels with factors group, caffeine and stage (baseline and finish line) and then gender, caffeine and stage, with respondents nested firstly in group (p = 0.56) and then in gender (p = 0.22). Similar analyses were done for red blood cell count, nested firstly in group (p = 0.23) and then in gender (p = 0.22); haemoglobin, nested firstly in group (p = 0.06) and then in gender (p = 0.50); heamatocrit, nested firstly in group (p = 0.23) and then in gender (p = 0.29); and platelet count, nested firstly in group (p = 0.33) and then in gender (p = 0.68). The following section explains the results of this analysis.

The red blood cell count in the caffeine group decreased significantly from baseline to the finish line in all subjects (p = 0.00*), and the male (p = 0.04*) and female (p = 0.00*) groups. The red blood cell count in the placebo group decreased significantly from baseline to the finish line in all subjects (p = 0.04*) and the male group (p = 0.03*), but not in the female group (p = 0.43). These differences were not due to caffeine supplementation (p = 0.25) and there were no differences between gender (p = 0.96).

Haemoglobin levels in the caffeine group at baseline and at the finish line did not differ significantly in all subjects (p = 0.29), and the male (p = 0.39) and female (p = 0.29) groups. Haemoglobin levels in the placebo group did not differ significantly at baseline and at the finish line in all subjects (p = 0.55), and the male (p = 0.44) and female (p = 0.92) groups. There was no difference in haemoglobin levels at baseline and at the finish line between the caffeine and placebo groups (p = 0.74), nor between gender (p = 0.97).

Heamatocrit levels in the caffeine group decreased significantly from baseline to the finish line in all subjects (p = 0.00*), and the male (p = 0.05*) and female (p = 0.00*) groups. Heamatocrit levels in the placebo group also decreased significantly from baseline to the finish line in the male group (p = 0.00*), but not in all subjects (p = 0.10) or in the female group (p = 0.50). These decreases were not due to caffeine supplementation (p = 0.18). Heamatocrit levels did not differ significantly between males and females (p = 0.91).

The platelet count in the caffeine and placebo groups increased significantly from baseline to the finish line in all subjects ($p = 0.00^*$), and in the male ($p = 0.00^*$) and female ($p = 0.00^*$) groups. This increase was not due to caffeine supplementation ($p = 0.50$). Platelet counts did not differ significantly between males and females ($p = 0.82$).

4.5.3 Mood state

The POMS questionnaire was completed during the week before T1 and T2, at baseline the morning of T1 and T2, as well as at the finish line after T1 and T2. There were no differences in the total POMS scores, tension or vigour scores measured at all the time points in the male and female groups (Table 4.15). There were however significantly higher fatigue scores in the week before T1 vs. T2 in the male group, and significantly lower fatigue scores in the week before T1 vs. T2 in the female group (Figure 4.20). These values changed at baseline and there were no differences observed in the fatigue scores measured at baseline in males and females before T1 or T2. The fatigue scores were higher in the male group when measured at the finish line, and lower in the female group, when compared to baseline, but these differences were not statistically significant.

Table 4.15 POMS scores the week before, at baseline and the finish line of T1 and T2*

	All ($N = 26$)		Males ($N_m = 14$)		Females ($N_f = 12$)		
	T1	T2	T1	T2	T1	T2	T1 vs. T2
Week before	Mean±SD	Mean±SD	Mean±SD	Mean±SD	Mean±SD	Mean±SD	p-value*
Total POMS score	-13.2±8.1	-12.3±8.0	-14.6±8.1	-11.4±7.8	-11.7±8.3	-13.3±8.5	$p = 0.11$
Tension	-2.3±1.8	-2.9±1.9	-2.4±1.7	-3.1±1.4	-2.2±2.0	-2.6±2.4	$p = 0.28$
Vigor	17.8±3.9	16.3±3.4	18.6±3.9	16.3±2.9	16.9±3.7	16.4±4.1	$p = 0.27$
Fatigue	6.9±5.4	6.9±6.1	6.5±5.5 ^a	8.0±6.4 ^b	7.4±5.5 ^a	5.7±5.6 ^b	$p = 0.04^*$
Baseline	Mean±SD	Mean±SD	Mean±SD	Mean±SD	Mean±SD	Mean±SD	p-value*
Total POMS score	-15.2±8.9	-13.5±8.6	-16.6±7.5	-15.2±7.6	-13.4±10.5	-11.4±9.5	$p = 0.88$
Tension	-2.4±2.8	-2.7±1.9	-2.9±2.3	-2.8±1.4	-1.9±3.3	-2.5±2.4	$p = 0.49$
Vigor	16.8±5.1	15.3±5.8	18.5±3.7	17.1±5.2	14.8±5.9	13.2±6.0	$p = 0.94$
Fatigue	4.0±4.5	4.5±4.9	4.7±5.2	4.6±5.2	3.3±3.7	4.3±4.7	$p = 0.62$
Finish line	Mean±SD	Mean±SD	Mean±SD	Mean±SD	Mean±SD	Mean±SD	p-value*
Total POMS score	-5.6±10.8	-3.6±10.1	-8.9±13.1	-4.4±10.1	-1.8±5.7	-2.7±10.5	$p = 0.18$
Tension	-1.5±2.4	-0.8±2.1	-1.4±1.9	-0.9±2.0	-1.5±3.0	-0.8±2.4	$p = 0.93$
Vigor	16.0±6.6	13.9±5.8	18.8±6.4	16.1±6.0	12.8±5.5	11.3±4.6	$p = 0.55$
Fatigue	11.8±5.6	11.1±5.6	12.3±6.2	12.6±5.1	12.5±4.9	9.5±6.0	$p = 0.06$

^a differed significantly from ^b ($p < 0.05$)

*RMANOVA

N_m = total male sample; N_f = total female sample

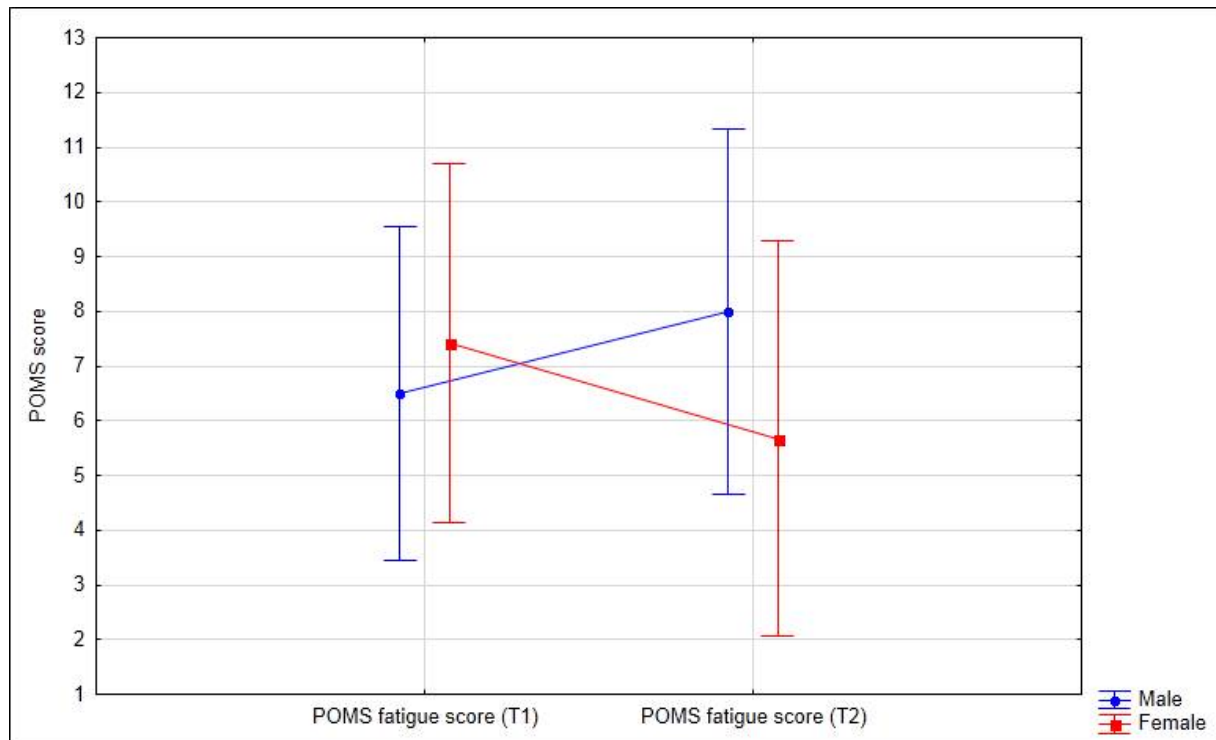


Figure 4.20 POMS fatigue score(s) during the week before T1 and T2 in males and females (Gender effect: $p = 0.04^*$)

The researcher used the Spearman rank-order correlation test to determine the effect of the total and differential POMS score during the week before T1 and T2, and at baseline and the effect of this on the overall time to complete the triathlons. The POMS scores measured during the week before and at baseline before T1 and T2 were able to influence the overall time to complete the triathlons. There were no significant correlations between the overall time to complete T1 and the total POMS score ($p = 0.28$), POMS tension score ($p = 0.17$), POMS vigour score ($p = 1.00$) or the POMS fatigue score ($p = 0.06$) during the week before T1. There was however a significant positive correlation between the total POMS score measured at baseline before T1 and the overall time to complete T1 ($p = 0.02^*$) (Figure 4.21). There were no relationship between the POMS tension ($p = 0.17$), POMS vigour ($p = 0.10$) and POMS fatigue ($p = 0.22$) scores measured at baseline before T1 and the overall time to complete T1.

Likewise, there were no significant correlations between the overall time to complete T2 and the total POMS score ($p = 0.33$), POMS tension score ($p = 0.42$), POMS vigour score ($p = 0.67$) or the POMS fatigue score ($p = 0.39$) during the week before T2. There were no significant correlations between the total POMS score ($p = 0.38$), POMS tension ($p = 0.47$), POMS vigour ($p = 0.80$) and POMS fatigue ($p = 0.14$) scores measured at baseline before T2 and the overall time to complete T2.

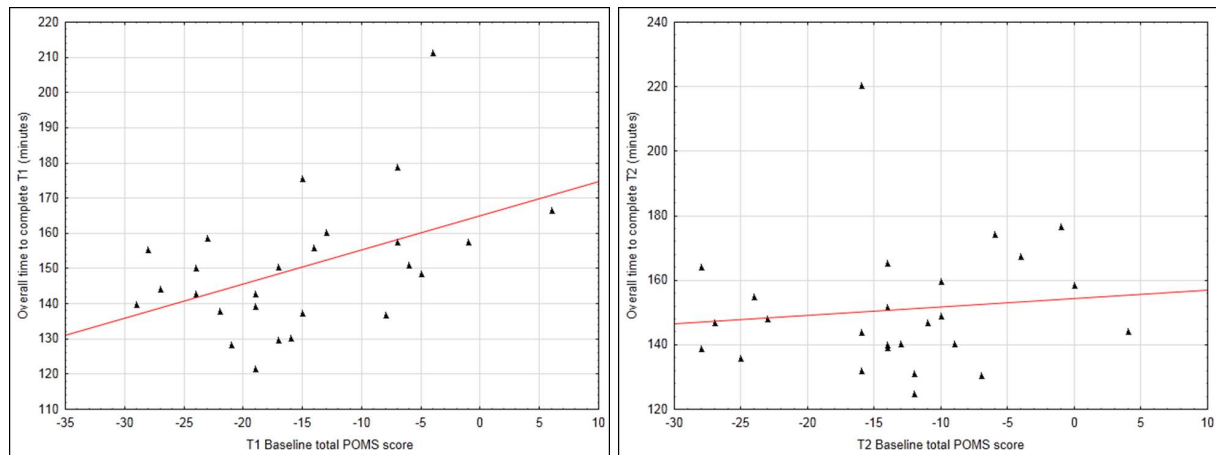


Figure 4.21 Relationship between total POMS scores measured at baseline and the overall time to complete T1 ($p = 0.02^*$) and T2 ($p = 0.38$) (minutes)

The researcher used the Spearman rank order correlation test to determine the effect of the total and differential POMS scores at baseline and the effect of these scores on the time to complete the swim sections of T1 and T2. The POMS scores measured at baseline before T1 and T2 were able to influence the time to complete the swim sections of T1 and T2. There were no significant correlations between the time to complete the swim section of T1 (minutes) and the total POMS score ($p = 0.08$), POMS tension score ($p = 0.09$), POMS vigour score ($p = 0.41$) or the POMS fatigue score ($p = 0.33$) measured at baseline the morning before T1. There were also no significant correlations between the time to complete the swim section of T2 (minutes) and the total POMS score ($p = 0.49$), POMS tension score ($p = 0.17$), POMS vigour score ($p = 0.86$) or the POMS fatigue score ($p = 0.69$) measured at baseline the morning before T2.

4.5.4 Dietary intake

4.5.4.1 Dietary intake two days before triathlon 1 (T1) and triathlon 2 (T2)

A Wilcoxon matched pairs test was completed to determine differences between the dietary intake two days before T1 and T2. As illustrated in Table 4.16, there was no significant difference in the dietary intake of the subjects two days before T1 and T2, except for alcohol intake, which was significantly higher two days before T2 in the whole group ($p = 0.05^*$) and in the male group ($p = 0.02^*$). It was concerning to note that amongst the total group the energy intake (36.5 ± 17.6 and 38.9 ± 18.2), $_{\text{est}}\text{EA}$ (27.9 ± 28.0 and 28.8 ± 25.6) and CHO intake (4.1 ± 1.6 g/kg BW and 4.6 ± 2.5 g/kg BW) before T1 and T2 respectively, were below the recommendations.

Table 4.16 Dietary intake two days before T1 and T2

	Recommendation	T1	T2	T1 vs. T2
All (N = 26)		Mean±SD	Mean±SD	p-value
Total energy intake (kcal/day)	2500-8000 kcal/day	2472.1±1034.5	2641.2±1119.0	p = 0.51
Total energy intake (kcal/kg BW/day)	50-80 kcal/kg BW/day (Moderate levels of intense training 2-3 hours/day, 5-6 X week)	36.5±17.6	38.9±18.2	p = 0.51
^{est} EA (kcal/kg FFM)	30-45 kcal/kg FFM	27.9±28.0	28.8±25.6	p = 0.90
Protein intake (g/kg BW/day)	1.0-1.5 g/kg BW	1.6±0.9	1.7±1.6	p = 0.97
CHO intake (g/kg BW/day)	6-10 g/kg BW (Endurance program, moderate- high intensity, 1-3 hours/day) 7-12 g/kg BW (General fuelling up (CHO-loading) for events > 90 minutes)	4.1±1.6	4.6±2.5	p = 0.28
Fat intake (g/kg BW /day)	0.8-1.5 g/kg BW	1.4±1.1	1.2±0.5	p = 0.95
Alcohol intake (g/day)	10-20 g/day	5.9±10.6 ^a	11.0±14.2 ^b	p = 0.05*
Fibre intake (g/day)	20-30 g/day	22.7±7.9	25.4±10.2	p = 0.14
Calcium (mg/day)	1000 mg/day (RDA/AI) – 2500 mg/day (UL)	1051.8±1258.9 (105% of DRI)	1571.8±3630.2 (157% of DRI)	p = 0.34
Calcium: Protein ratio	20 mg calcium per 1 g protein	9.5±4.9	10.7±6.8	p = 0.41
Iron (mg/day)	Male & female reference values	16.6±11.6	17.6±8.8	p = 0.09
Male (N_m = 14)		Mean±SD	Mean±SD	p-value
Total energy intake (kcal/day)	2500-8000 kcal/day	2520.9±708.8	2692.3±701.8	p = 0.28
Total energy intake (kcal/kg BW/day)	50-80 kcal/kg BW/day	33.8±9.5	35.9±7.6	p = 0.28
^{est} EA (kcal/kg FFM)	30-45 kcal/kg FFM	20.8±14.2	24.7±19.7	p = 0.55
Protein intake (g/kg BW/day)	1.0-1.5 g/kg BW	1.4±0.6	1.4±0.4	p = 0.65
CHO intake (g/kg BW/day)	6-10 g/kg BW or 7-12 g/kg BW	3.9±1.5	3.9±1.2	p = 0.20
Fat intake (g/kg BW /day)	0.8-1.5 g/kg BW	1.1±0.3	1.2±0.3	p = 0.31
Alcohol intake (g/day)	10-20 g/day	7.4±12.1 ^a	17.2±16.9 ^b	p = 0.02*
Fibre intake (g/day)	20-30 g/day	23.1±6.5	27.6±11.3	p = 0.18
Calcium (mg/day)	1000 mg/day (RDA/AI) – 2500 mg/day (UL)	844.0±377.9	958.3±446.8	p = 0.15
Calcium: Protein ratio	20 mg calcium per 1 g protein	8.7±4.4	9.3±4.6	p = 0.65
Iron (mg/day)	6-8 mg/day (EAR-RDA/AI), - 45 mg/day (UL)	19.5±14.4 (244% of RDA)	20.5±10.7 (256% of RDA)	p = 0.13
Female (N_f = 12)		Mean±SD	Mean±SD	p-value
Total energy intake (kcal/day)	2500-8000 kcal/day	2415.1±1353.5	2585.9±1479.3	p = 0.88
Total energy intake (kcal/kg BW/day)	50-80 kcal/kg BW/day	39.6±24.1	42.1±25.2	p = 0.94
^{est} EA (kcal/kg FFM)	30-45 kcal/kg FFM	36.3±37.4	33.5±31.4	p = 0.82
Protein intake (g/kg BW/day)	1.0-1.5 g/kg BW	1.7±1.2	2.0±2.3	p = 0.69
CHO intake (g/kg BW/day)	6-10 g/kg BW or 7-12 g/kg BW	4.4±1.7	5.3±3.3	p = 0.69
Fat intake (g/kg BW /day)	0.8-1.5 g/kg BW	1.6±1.6	1.2±0.6	p = 0.43
Alcohol intake (g/day)	10-20 g/day	4.2±8.8	4.3±5.9	p = 1.00
Fibre intake (g/day)	20-30 g/day	22.3±9.6	23.0±8.8	p = 0.50
Calcium (mg/day)	1000 mg/day (RDA/AI) – 2500 mg/day (UL)	1294.3±1820.5	2236.5±5254.3	p = 0.94
Calcium: Protein ratio	20 mg calcium per 1 g protein	10.5±5.4	12.3±8.5	p = 0.53
Iron (mg/day)	8.1-18 mg/day (EAR-RDA/AI), - 45 mg/day (UL)	13.2±5.9 (73% of RDA)	14.5±6.5 (81% of RDA)	p = 0.39

Sources: (4, 169, 170, 172, 174, 175, 183, 203)

^a differed significantly from ^b (p < 0.05)kcal: calories; kcal/kg BW/day: calories per kilogram body weight per day; kcal/kg FFM: calories per kilogram fat free mass; g/kg BW: gram per kilogram body weight; TE: total energy; EAR: Estimated Average Requirement; RDA: Recommended Dietary Allowance; AI: Adequate Intake; UL: Upper Limit; N = total sample; N_m = total male sample; N_f = total female sample

Although the alcohol intake differed significantly two days before T1 and T2 in the whole group and in the male group, it did not influence the overall time to complete T1 (p = 0.64

and $p = 0.43$ respectively) or T2 ($p = 0.81$ and $p = 0.40$ respectively). However, a higher fibre intake was associated with a shorter/decreased overall time to complete T1 ($p = 0.02^*$) and T2 ($p = 0.04^*$) (Figure 4.22) and an increased CHO intake two days before T2 in the male group significantly decreased the overall time to complete T2 in this group ($p = 0.05^*$) (Figure 4.23).

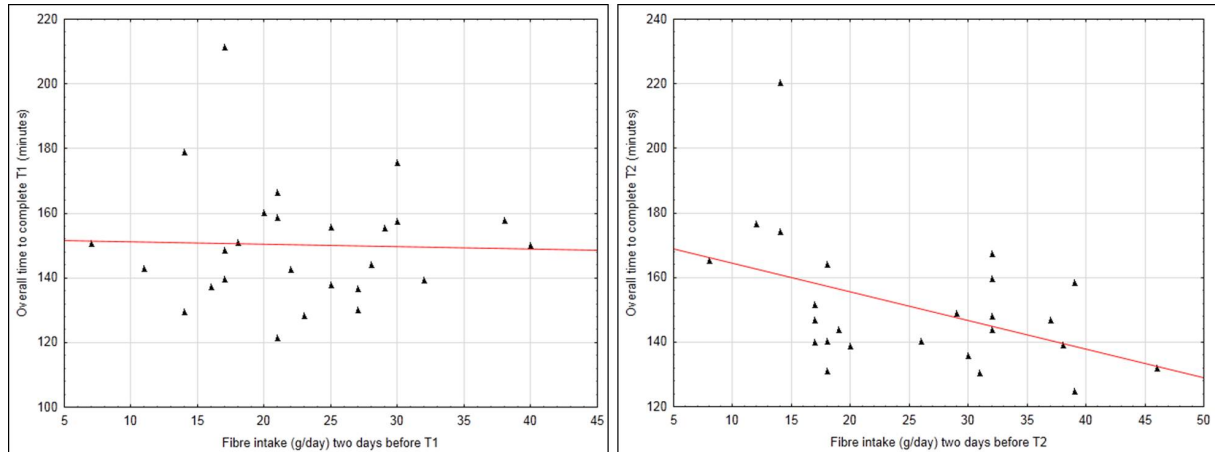


Figure 4.22 Relationship between the overall time to complete and the total fibre intake (g/day) two days before T1 ($p = 0.02^*$) and T2 ($p = 0.04^*$) in the whole group ($N = 26$) as determined by the Spearman R rank-order correlation.

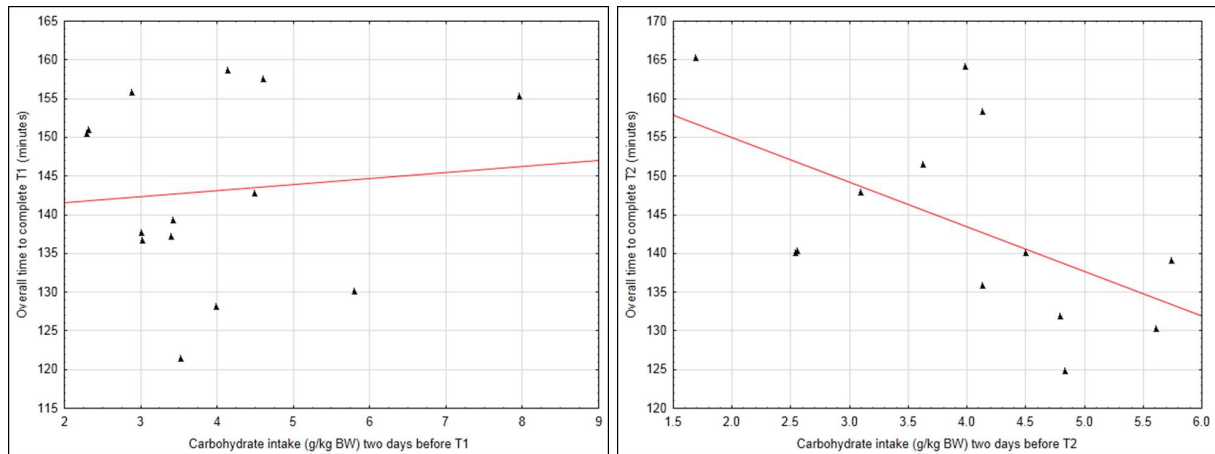


Figure 4.23 Relationship between the overall time to complete and the total CHO intake (g/kg body weight (BW)) two days before T1 ($p = 0.74$) and T2 ($p = 0.05^*$) in the male group ($N_m = 14$) as determined by the Spearman R rank-order correlation.

The mean number of meals (meal frequency) of the subjects two days before T1 and T2 (data combined) was $6.7 (\pm 2.2)$ meals. The top 15 food choices include the following: oats (77.4 ± 60.3 g), Rooibos tea (267.8 ± 50.7 ml), brown bread (71.1 ± 35.3 g), low-fat milk or yogurt (108.1 ± 96.0 ml), chicken (112.7 ± 83.5 g), peanut butter (18.8 ± 11.7 g), tuna (96.8 ± 57.5

g), avocado pear (80.8 ± 62.8 g), fruit (apples (130.7 ± 45.8 g) and bananas (96.6 ± 21.0 g)), red meat (beef) (153.9 ± 106.9 g), eggs (2.1 ± 0.7 eggs), honey (17.6 ± 8.6 g), spaghetti bolognaise (259.1 ± 119.6 g), rusks (40.3 ± 24.5 g) and pizza (347.2 ± 195.3 g). The food groups most athletes chose from included the starch, protein (including milk and dairy) and fat (unsaturated) groups.

The combined (T1 and T2) estEA of the subjects were $25.5 (\pm 22.9)$ for all; and $17.9 (\pm 10.7)$ and $34.3 (\pm 30.0)$ for males and females, respectively, which is low.

4.5.4.2 Pre-event dietary intake before T1 and T2

A Wilcoxon matched pairs test was used to determine differences between pre-event dietary intakes on the morning of T1 and T2. There were no significant differences in the dietary intake of the subjects on the morning of the race between T1 and T2, except for higher total energy intake ($p = 0.04^*$) in the females and higher fat intake (g) ($p = 0.04^*$) in the whole subject group prior to T1 when compared to T2 (Table 4.16). Carbohydrate is the most important nutrient in the pre-event meal. In the present study, the pre-event meal was not adequate in terms of carbohydrate content (0.7 ± 0.4 and 0.7 ± 0.5 g/kg BW) (Table 4.17).

Table 4.17 Dietary intake the morning of T1 and T2 (i.e. the pre-event meal)

	Recommendation	T1	T2	T1 vs. T2
All (N = 26)		Mean±SD	Mean±SD	p-value
Total energy intake (kcal)	N/A	377.1±242.5	298.8±230.7	p = 0.26
Total energy intake (kcal/kg BW)	N/A	4.7±3.3	4.2±2.9	p = 0.22
Protein intake (g/kg BW)	0.15-0.25 g/kg BW*	0.1±0.1	0.1±0.1	p = 0.20
CHO intake (g/kg BW)	1-4 g/kg BW (Pre-event fuelling, exercise > 60 minutes)	0.7±0.4	0.7±0.5	p = 0.74
Fat intake (g)	N/A	10.0±11.2 ^a	7.4±10.5 ^b	p = 0.04*
Fat intake (g/kg BW)	N/A	0.1±0.2	0.1±0.1	p = 0.95
Male (N_m = 14)		Mean±SD	Mean±SD	p-value
Total energy intake (kcal)	N/A	418.3±259.6	386.1±256.9	p = 0.69
Total energy intake (kcal/kg BW)	N/A	5.5±3.4	5.1±3.1	p = 0.64
Protein intake (g/kg BW)	0.15-0.25 g/kg BW*	0.2±0.1	0.1±0.1	p = 0.16
CHO intake (g/kg BW)	1-4 g/kg BW	0.8±0.4	0.8±0.5	p = 0.64
Fat intake (g)	N/A	12.8±13.1	9.4±13.5	p = 0.06
Fat intake (g/kg BW)	N/A	0.2±0.2	0.1±0.2	p = 0.31
Female (N_f = 12)		Mean±SD	Mean±SD	p-value
Total energy intake (kcal)	N/A	227.5±185.4 ^a	195.7±147.0 ^b	p = 0.04*
Total energy intake (kcal/kg BW)	N/A	3.7±3.1	3.2±2.5	p = 0.07
Protein intake (g/kg BW)	0.15-0.25 g/kg BW*	0.1±0.1	0.1±0.1	p = 0.89
CHO intake (g/kg BW)	1-4 g/kg BW	0.5±0.4	0.5±0.4	p = 0.21
Fat intake (g)	N/A	6.9±8.2	5.1±4.8	p = 0.40
Fat intake (g/kg BW)	N/A	0.1±0.1	0.1±0.1	p = 0.43

Sources: (4, 169, 170, 172, 174, 175, 183, 203)

^a differed significantly from ^b (p < 0.05)kcal: calories; kcal/kg BW/day: calories per kilogram body weight per day; kcal/kg FFM: calories per kilogram fat free mass; g/kg BW: gram per kilogram body weight; N/A: not applicable; N = total sample; N_m = total male sample; N_f = total female sample

Although the total energy content (kcal/meal) of the pre-event meal in the female group differed significantly between T1 and T2 it did not influence the overall time to complete T1 and T2 in the female group (p = 0.10).

However, as expected the total energy content of the pre-event meal (kcal/meal) inversely correlated with the overall time to complete T1 and T2 in the whole group (p = 0.02* and p = 0.02* respectively) (Figure 4.24).

The CHO content (g/kg BW) of the pre-event meal in the whole group also showed a negative correlation with the overall time to complete T1 and T2 (p = 0.00* and p = 0.00* respectively) (Figure 4.25).

Although the fat intake in the whole group differed significantly between T1 and T2 it did not seem to influence the overall time to complete T1 and T2 (p = 0.22 and p = 0.54 respectively).

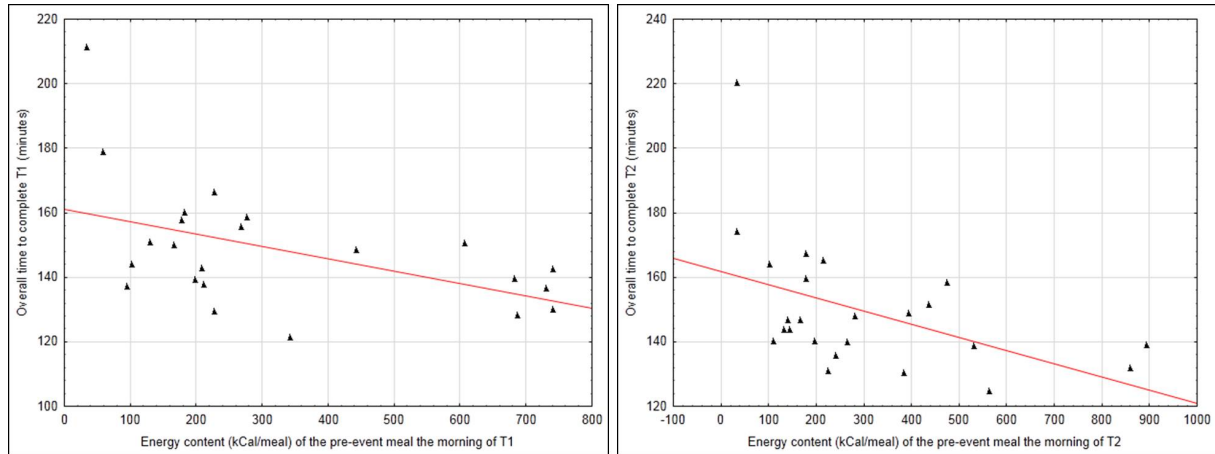


Figure 4.24 Relationship between the overall time to complete and the total energy content (kcal/meal) of the pre-event meal the morning of T1 ($p = 0.02^*$) and T2 ($p = 0.02^*$) in the whole group ($N = 26$) as determined by the Spearman R rank-order correlation.

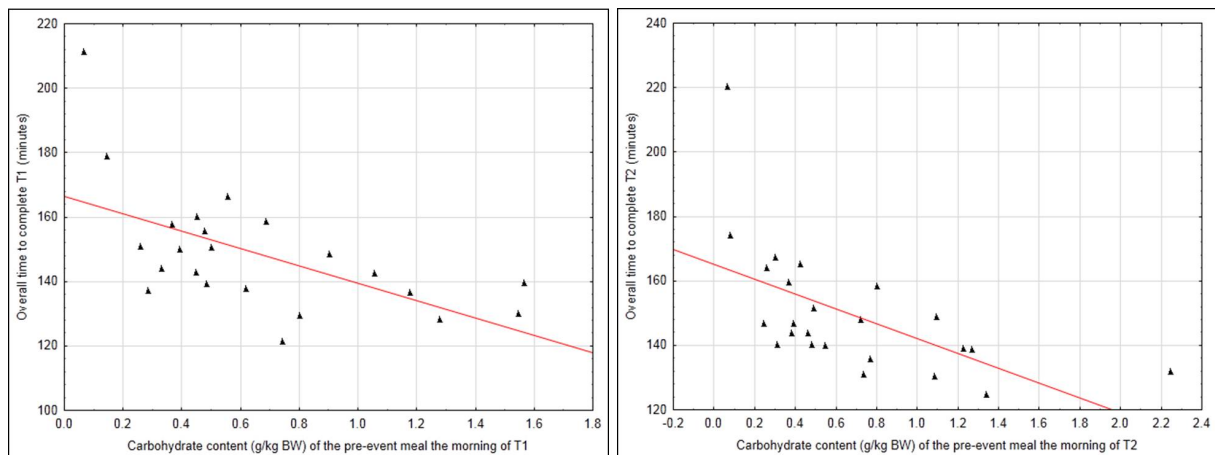


Figure 4.25 Relationship between the overall time to complete and the CHO content (g/kg BW) of the pre-event meal the morning of T1 ($p = 0.00^*$) and T2 ($p = 0.00^*$) in the whole group ($N = 26$) as determined by the Spearman R rank-order correlation.

Most of the subjects ($N = 22$) ate a pre-event meal before T1 and T2 (combined data). The most commonly consumed meals were oats (68.2 ± 57.8 g) and brown bread (60.5 ± 21.4 g) with peanut butter (33.3 ± 22.5 g) and banana (108.3 ± 45.6 g). The most commonly consumed drink was rooibos tea (250.0 ± 26.7 ml)) and their carbohydrate-electrolyte solution of choice (215.0 ± 105.5 ml), which included, but is not limited to, Energade[®], PVM Octane[®] or 32 GI[®].

4.5.4.3 Dietary intake during T1 and T2

A Wilcoxon matched pairs test was used to determine differences between dietary intake during T1 and T2. There were no significant difference in the dietary intake of the subjects during T1 and T2, except for total energy ($p = 0.02^*$) and CHO intake ($p = 0.02^*$) in male subjects (Table 4.18), which was higher during T1 when compared to T2 (total energy intake; 488.5 ± 721.3 vs. 208.8 ± 105.6 kcal and CHO intake; 1.6 ± 2.3 vs. 0.7 ± 0.4 g/kg BW).

Table 4.18 Dietary intake during T1 and T2

	Recommendation	T1	T2	T1 vs. T2
All ($N = 26$)		Mean\pmSD	Mean\pmSD	p-value
Total energy intake (kcal)	N/A	467.9 \pm 613.7	262.6 \pm 138.0	$p = 0.13$
Total energy intake (kcal/kgBW)	N/A	6.8 \pm 8.6	4.0 \pm 2.4	$p = 0.15$
Protein intake (g/kg BW)	N/A	0.1 \pm 0.1	0.0 \pm 0.1	$p = 0.21$
CHO intake (g/kg BW)	0.7 g/kg BW CHO (ACSM) 30-60 g/hour*	1.6 \pm 2.3	0.9 \pm 0.5	$p = 0.16$
Fat intake (g/kg BW)	N/A	1.5 \pm 2.8	0.9 \pm 2.0	$p = 0.17$
CHO: Protein ratio	CHO:Protein ratio of 3-4:1	10.8 \pm 8.2	24.7 \pm 42.7	$p = 0.50$
Male ($N_m = 14$)		Mean\pmSD	Mean\pmSD	p-value
Total energy intake (kcal)	N/A	488.5 \pm 721.3 ^a	208.8 \pm 105.6 ^b	$p = 0.02^*$
Total energy intake (kcal/kgBW)	N/A	6.2 \pm 8.5 ^a	2.8 \pm 1.5 ^b	$p = 0.02^*$
Protein intake (g/kg BW)	N/A	0.0 \pm 0.0	0.0 \pm 0.0	$p = 0.29$
CHO intake (g/kg BW)	0.7 g/kg BW CHO (ACSM) 30-60 g/hour*	1.6 \pm 2.3 ^a	0.7 \pm 0.4 ^b	$p = 0.02^*$
Fat intake (g/kg BW)	N/A	0.7 \pm 2.1	0.3 \pm 1.0	$p = 0.65$
CHO: Protein ratio	CHO:Protein ratio of 3-4:1	9.9 \pm 9.2	9.0 \pm 6.3	$p = 0.72$
Female ($N_f = 12$)		Mean\pmSD	Mean\pmSD	p-value
Total energy intake (kcal)	N/A	449.1 \pm 533.0	321.7 \pm 150.1	$p = 0.96$
Total energy intake (kcal/kgBW)	N/A	7.4 \pm 9.1	5.3 \pm 2.6	$p = 0.96$
Protein intake (g/kg BW)	N/A	0.1 \pm 0.1	0.1 \pm 0.1	$p = 0.50$
CHO intake (g/kg BW)	0.7 g/kg BW CHO (ACSM) 30-60 g/hour*	1.6 \pm 2.4	1.1 \pm 0.6	$p = 0.96$
Fat intake (g/kg BW)	N/A	2.3 \pm 3.2	1.6 \pm 2.6	$p = 0.27$
CHO: Protein ratio	CHO:Protein ratio of 3-4:1	9.9 \pm 9.2	9.0 \pm 6.3	$p = 0.72$

Sources: (4, 169, 170, 172, 174, 175, 183, 203)

^a differed significantly from ^b ($p < 0.05$)

kcal: calories; kcal/kgBW/day: calories per kilogram body weight per day; kcal/kgFFM: calories per kilogram fat free mass; g/kg BW: gram per kilogram body weight; N = total sample; N_m = total male sample; N_f = total female sample

Although the total energy (kcal) and CHO intake (g/kg BW) during T1 and T2 differed significantly in the male group it did not show any correlation with the overall time to complete T1 and T2 ($p = 0.55$ and $p = 0.10$) as determined by the Spearman R rank-order correlation.

Only 16 of the subjects ingested a food or drink during the triathlon (when combining data from T1 and T2). Most of the subjects ingested a form of carbohydrate-electrolyte solution (539.5 ± 178.3 ml), such as Energade[®], PVM Octane[®] or 32 GI[®]. Subjects also chose PVM[®]

energy bars (32.5 ± 11.5 g)) and GU[®] energy gels (35.4 ± 17.1 g) as food to be consumed during the triathlons.

4.5.5 Body composition and anthropometry

4.5.5.1 Bone densitometry

Bone densitometry was determined by means of DXA. It was alarming to note that in the female group, the whole body BMD revealed that 72% ($N_{f \text{ pre-men}} = 5$) and 40% ($N_{f \text{ post-men}} = 2$) was classified as low BMD (Z-scores used according to ACSM criteria for pre-menopausal women) and osteopenic (T-scores used according to WHO criteria for post-menopausal women) respectively. It was also noted that according to this whole body classification, 14% ($N_{f \text{ pre-men}} = 1$) and 20% ($N_{f \text{ post-men}} = 1$) was classified as osteoporotic according to above mentioned classification systems (Table 4.19).

There were no statistically significant correlations found between BMD and the following parameters in all, males and females respectively: estEA ($p = 0.53$, $p = 0.29$, $p = 0.90$), BMI ($p = 0.54$, $p = 0.49$, $p = 0.57$), body weight ($p = 0.09$, $p = 0.20$, $p = 0.56$), age ($p = 0.40$, $p = 0.31$, $p = 0.92$) and height in males ($p = 0.22$) and females ($p = 0.87$).

There was however, a significant positive correlation between BMD and height in the total subject group ($p = 0.04^*$) (Figure 4.26).

Table 4.19 Bone densitometry

	N_m < 50 years = 11	N_m > 50 years = 3	N_f pre-men = 7	N_f post-men = 5
Anterior-posterior spine:	Mean±SD	Mean±SD	Mean±SD	Mean±SD
Bone mineral density (g/cm ²)	1.1±0.1	1.0±0.1	1.0±0.1	1.1±0.2
T-score		-0.7±1.0		0.1±2.1
Z-score	-0.2±1.2		-0.6±1.0	
Classification*	% (N _m < 50 years)	% (N _m > 50 years)	% (N _f pre-men)	% (N _f post-men)
Normal	82% (9)	67% (2)	57% (4)	60% (3)
Osteopenic (WHO), Low BMD (ACSM)	18% (2)	33% (1)	43% (3)	40% (2)
Osteoporotic	0% (0)	0% (0)	0% (0)	0% (0)
Left femoral neck:	Mean±SD	Mean±SD	Mean±SD	Mean±SD
Bone mineral density (g/cm ²)	0.9±0.1	0.8±0.0	0.8±0.1	0.8±0.1
T-score		-0.8±0.4		-0.0±1.0
Z-score	0.0±0.9		-0.1±0.6	
Classification*	% (N _m < 50 years)	% (N _m > 50 years)	% (N _f pre-men)	% (N _f post-men)
Normal	100% (11)	67% (2)	86% (6)	80% (4)
Osteopenic (WHO), Low BMD (ACSM)	0% (0)	33% (1)	14% (1)	20% (1)
Osteoporotic	0% (0)	0% (0)	0% (0)	0% (0)
Left total hip:	Mean±SD	Mean±SD	Mean±SD	Mean±SD
Bone mineral density (g/cm ²)	1.0±0.1	1.0±0.0	1.0±0.1	1.0±0.1
T-score		0.1±0.3		0.2±0.9
Z-score	0.1±0.7		0.2±0.6	
Classification*	% (N _m < 50 years)	% (N _m > 50 years)	% (N _f pre-men)	% (N _f post-men)
Normal	100% (11)	100% (3)	100% (7)	100% (5)
Osteopenic (WHO), Low BMD (ACSM)	0% (0)	0% (0)	0% (0)	0% (0)
Osteoporotic	0% (0)	0% (0)	0% (0)	0% (0)
Total hip bilateral average:	Mean±SD	Mean±SD	Mean±SD	Mean±SD
Bone mineral density (g/cm ²)	1.0±0.1	1.0±0.1	1.0±0.1	1.0±0.1
T-score		0.0±0.4		0.1±1.0
Z-score	0.0±0.7		0.1±0.7	
Classification*	% (N _m < 50 years)	% (N _m > 50 years)	% (N _f pre-men)	% (N _f post-men)
Normal	100% (11)	100% (3)	86% (6)	100% (5)
Osteopenic (WHO), Low BMD (ACSM)	0% (0)	0% (0)	14% (1)	0% (0)
Osteoporotic	0% (0)	0% (0)	0% (0)	0% (0)
Whole body:	Mean±SD	Mean±SD	Mean±SD	Mean±SD
Bone mineral density (g/cm ²)	1.1±0.1	1.1±0.1	1.0±0.1	1.0±0.1
T-score		-1.1±0.6		-1.3±1.2
Z-score	0.0±0.0		-1.1±1.1	
Classification*	% (N _m < 50 years)	% (N _m > 50 years)	% (N _f pre-men)	% (N _f post-men)
Normal	100% (11)	67% (2)	14% (1)	40% (2)
Osteopenic (WHO), Low BMD (ACSM)	0% (0)	33% (1)	72% (5)	40% (2)
Osteoporotic	0% (0)	0% (0)	14% (1)	20% (1)
Bone mineral content (g)	Mean±SD	Mean±SD	Mean±SD	Mean±SD
Total	2451.4±444.2	2543.53.5	1925.0±239.2	2020.6±258.4

*Classification for pre-menopausal women and men < 50 years based on ACSM criteria (Z-scores), classification for post-menopausal women and men > 50 years based on WHO criteria (T-scores).

N = total sample; N_m < 50 years = total male sample < 50 years; N_m > 50 years = total male sample > 50 years; N_f pre-men = total female sample pre-menopause; N_f post-men = total female sample post-menopause

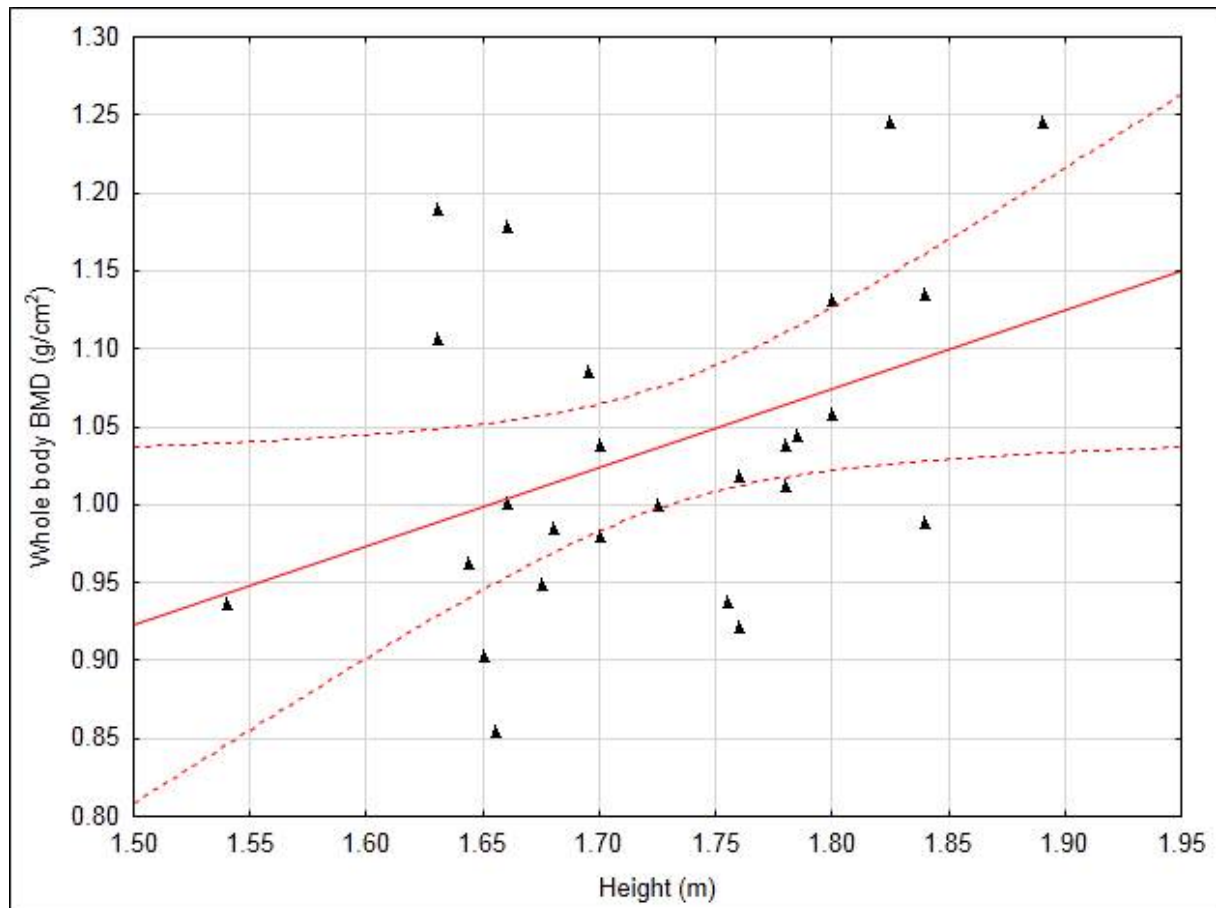


Figure 4.26 Relationship between whole body BMD (g/cm²) and height (m) in the total subject group ($p = 0.04^*$)

4.5.5.2 Body composition and anthropometry

Table 4.20 summarizes the body composition and anthropometrical measurements of the triathletes. The ACSM recommends that triathletes should aim for a percentage body fat of between 6-15%, males aiming for the lower and females for the upper range. The current study population falls within this range ($14.5 \pm 7.2\%$), with the males $10.7 \pm 3.8\%$ and females above the recommendation at $19.0 \pm 7.7\%$.

Table 4.20 Body composition measurements and anthropometry

	All (N = 26)	Male (N _m = 14)	Female (N _f = 12)
Body composition ^a	Mean±SD	Mean±SD	Mean±SD
Percentage body fat (%)	14.5±7.2	10.7±3.8	19.0±7.7
Fat (kg)	9.5±5.0	7.7±3.2	11.5±5.9
Lean body mass (LBM) (kg)	53.8±9.6	61.0±6.5	45.4±4.0
Fat free mass (lean + BMC) (kg)	56.±9.9	63.5±6.8	47.4±4.1
Body mass (kg)	65.5±10.0	71.2±8.4	58.9±7.5
Anthropometry ^b	Mean±SD	Mean±SD	Mean±SD
Height (cm)	172.5±8.3	178.0±6.2	166.2±5.4
Body weight (kg)	69.2±10.6	75.3±8.8	62.2±7.8
BMI (kg/m ²)	21.9±2.0	22.4±1.8	21.3±2.1

^aMeasured with DXA^bMeasured with Seca® 767 column scale with height meterN = total sample; N_m = total male sample; N_f = total female sampleBMI: Body mass index (kg/m²)

4.5.6 Training regime

4.5.6.1 Usual training habits / regime

The workloads for exercise sessions completed per week by the subjects as part of their usual training regime are presented in Table 4.21. While males and females spent similar times training for swimming and cycling, females spent significantly more time running than males (4.3 (±1.3) vs. 2.8 (±1.2) hours per week) (p = 0.01*). The average amount of training per week was 12.8 (±4.8) hours for the whole group.

Table 4.21 Usual training regime

	All (N = 26)	Male (N _m = 14)	Female (N _f = 12)	Male vs. Female
	Mean±SD	Mean±SD	Mean±SD	p-value
Exercise sessions / week	6.5±0.5	6.1±3.1	7.1±2.1	p = 0.35
Exercise sessions / day	1.5±2.7	1.4±0.5	1.7±0.5	p = 0.14
Swimming / week (hours)	2.5±1.2	2.1±1.3	2.9±1.0	p = 0.10
Swimming / week (km)	5.8±3.9	5.6±4.5	6.0±3.4	p = 0.80
Cycling / week (hours)	5.3±2.2	5.3±2.5	5.3±2.0	p = 1.00
Cycling / week (km)	153.1±103.0	166.8±122.7	137.1±76.0	p = 0.47
Running / week (hours)	3.5±1.4	2.8±1.2 ^a	4.3±1.3 ^b	p = 0.01*
Running / week (km)	37.1±15.0	30.7±13.1 ^a	44.6±14.0 ^b	p = 0.02*
Gym / week (hours)	0.9±1.1	0.8±0.8	1.0±1.5	p = 0.67
Other / week (hours) ^a	0.9±1.2	1.1±1.2	0.6±1.1	p = 0.28
OD tri's completed in 2010/11	4.5±4.7	4.7±6.4	4.3±1.8	p = 0.84
OD tri PB (minutes)	144.8±18.3	139.0±14.0	153.0±21.7	p = 0.06
OD tri PB (hours)	2h25m±18.3m	2h19m±14.0	2h33m±21.7	p = 0.06

^a differed significantly from ^b (p < 0.05)km: kilometers; OD: Olympic-distance; tri: triathlon; PB: personal best; ^aOther: includes aerobic exercise other than running; swimming and cycling (e.g. rowing; canoeing); N = total sample; N_m = total male sample; N_f = total female sample

4.5.6.2 Training characteristics two (2) days before T1 and T2

As determined with the t-test for independent samples, there were no differences in the type and volume of training completed before T1 vs. T2 (Table 4.22). However, the rating of exertion in running was significantly higher before T2, when compared to T1 in the male group ($p = 0.04^*$).

Table 4.22 Training completed two days before T1 and T2

	T1		T2		T1 vs. T2
All ($N = 26$)	<i>N</i>	Mean \pm SD	<i>N</i>	Mean \pm SD	p-value
Swimming (minutes)	13	35.7 \pm 11.5	12	35.8 \pm 19.0	$p = 0.98$
Swimming (km)	13	1.6 \pm 0.5	12	1.6 \pm 0.9	$p = 0.96$
Swimming (exertion) ^c	13	3.1 \pm 0.8	13	3.1 \pm 0.9	$p = 0.98$
Cycling (minutes)	10	56.0 \pm 49.2	11	67.5 \pm 49.1	$p = 0.62$
Cycling (km)	10	26.2 \pm 25.0	11	27.0 \pm 18.6	$p = 0.94$
Cycling (exertion) ^c	10	2.6 \pm 0.5	11	3.0 \pm 0.8	$p = 0.24$
Running (minutes)	12	27.8 \pm 14.3	5	27.0 \pm 20.2	$p = 0.93$
Running (km)	12	5.3 \pm 2.7	5	4.8 \pm 2.9	$p = 0.72$
Running (exertion)	12	2.5 \pm 0.7	5	3.1 \pm 1.1	$p = 0.15$
Other (minutes) ^d	5	49.0 \pm 29.2	5	59.0 \pm 23.0	$p = 0.56$
Male ($N_m = 14$)	<i>N</i>	Mean \pm SD	<i>N</i>	Mean \pm SD	p-value
Swimming (minutes)	9	36.0 \pm 12.4	7	35.0 \pm 22.7	$p = 0.91$
Swimming (km)	9	1.5 \pm 0.6	7	1.5 \pm 0.9	$p = 0.89$
Swimming (exertion) ^c	9	3.4 \pm 0.7	7	3.6 \pm 0.8	$p = 0.74$
Cycling (minutes)	6	38.7 \pm 27.9	6	51.3 \pm 35.2	$p = 0.51$
Cycling (km)	6	18.2 \pm 14.1	6	22.0 \pm 12.4	$p = 0.63$
Cycling (exertion)	6	2.7 \pm 0.5	6	3.2 \pm 0.8	$p = 0.21$
Running (minutes)	8	26.9 \pm 15.6	3	28.3 \pm 28.4	$p = 0.91$
Running (km)	8	5.4 \pm 3.2	3	4.7 \pm 4.0	$p = 0.76$
Running (exertion)	8	2.5 \pm 0.5 ^a	3	3.7 \pm 1.2 ^b	$p = 0.04^*$
Other* (minutes) ^d	4	50.0 \pm 33.7	3	63.3 \pm 30.6	$p = 0.61$
Female ($N_f = 12$)	<i>N</i>	Mean \pm SD	<i>N</i>	Mean \pm SD	p-value
Swimming (minutes)	4	35.0 \pm 10.8	5	37.0 \pm 14.8	$p = 0.83$
Swimming (km)	4	1.6 \pm 0.6	5	1.7 \pm 0.8	$p = 0.86$
Swimming (exertion) ^c	4	2.4 \pm 0.5	5	2.5 \pm 0.5	$p = 0.72$
Cycling (minutes)	4	82.0 \pm 66.8	5	87.0 \pm 60.2	$p = 0.91$
Cycling (km)	4	38.3 \pm 34.9	5	33.0 \pm 24.2	$p = 0.80$
Cycling (exertion)	4	2.5 \pm 0.8	5	2.7 \pm 0.8	$p = 0.70$
Running (minutes)	4	29.5 \pm 13.2	2	25.0 \pm 0.0	$p = 0.67$
Running (km)	4	5.3 \pm 1.9	2	5.0 \pm 0.0	$p = 0.18$
Running (exertion)	4	2.5 \pm 1.0	2	2.3 \pm 0.4	$p = 0.33$
Other* (minutes) ^d	1	45.0 \pm 0.0	2	52.5 \pm 10.6	$p = 0.67$

^a differed significantly from ^b ($p < 0.05$)

^cExertion measured on a 5 point scale; ^dOther: includes aerobic exercise other than running; swimming and cycling (e.g. rowing; canoeing); *N* = total sample; *N_m* = total male sample; *N_f* = total female sample

Although the rating of exertion for running two days before racing was higher before T2, when compared to T1 in the male group it did not influence the overall time to complete T1 (143.0 \pm 12.0 minutes) or T2 (144.6 \pm 12.7 minutes) ($p = 0.45$ and $p = 0.60$ respectively) according to the Pearson correlation coefficient.

4.5.7 Caffeine withdrawal symptoms

A questionnaire was completed before T1 to determine any subjective caffeine withdrawal symptoms experienced during the first two weeks of caffeine abstinence. The most common withdrawal symptom experienced were headaches (46%, $N = 12$), followed by flu-like symptoms (38%, $N = 10$) (Table 4.23).

Table 4.23 Prevalence of caffeine withdrawal symptoms

	All ($N = 26$)	Male ($N_m = 14$)	Female ($N_f = 12$)
	% (N)	% (N_m)	% (N_f)
Headaches	46% (12)	21% (3)	75% (9)
Fatigue	31% (8)	14% (2)	50% (6)
Lethargy	35% (9)	21% (3)	50% (6)
Flu-like symptoms	38% (10)	14% (2)	67% (8)

N = total sample; N_m = total male sample; N_f = total female sample

According to the Mann-Whitney U test, headaches experienced in the two weeks before T1 increased the overall time to complete T1 in the male and female groups, as well as in the combined group (Table 4.24).

Table 4.24 Influence of headaches experienced during the two weeks before T1 on the overall time to complete T1

	Time to complete (minutes)		Yes vs. No
All ($N = 26$)	Mean \pm SD	N	p-value
Headaches (Y)	160.5 \pm 20.7 ^a	12	$p = 0.00^*$
Headaches (N)	141.5 \pm 11.7 ^b	14	
Male ($N_m = 14$)	Mean \pm SD	N	p-value
Headaches (Y)	146.4 \pm 10.9 ^a	3	$p = 0.05^*$
Headaches (N)	142.1 \pm 12.6 ^b	11	
Female ($N_f = 12$)	Mean \pm SD	N	p-value
Headaches (Y)	165.2 \pm 21.5 ^a	9	$p = 0.04^*$
Headaches (N)	139.3 \pm 9.4 ^b	3	

^a differed significantly from ^b ($p < 0.05$)

N = total sample; N_m = total male sample; N_f = total female sample; Y = yes; N = no

4.5.8 Side effects of caffeine supplementation

The subjects were asked to complete a subjective questionnaire to determine which of the most common side-effects of caffeine supplementation were experienced and how these may have affected the subject's overall time to complete the triathlon in the caffeine and placebo groups respectively. When comparing the caffeine and placebo groups with the M-L chi-squared test, the whole group experienced shakiness ($p = 0.00^*$), heart palpitations ($p = 0.01^*$) and GIT disturbances ($p = 0.01^*$) when receiving caffeine supplementation. In the

female group, shakiness ($p = 0.05^*$) and heart palpitations ($p = 0.05^*$) were more prevalent while GIT disturbances ($p = 0.01^*$) were more prevalent in the male caffeine vs. placebo group.

It is interesting to note that, although the values did not reach statistical significance, nervousness was less prevalent in both males (7% reduction) and females (25% reduction) in the caffeine group compared to the placebo group (Table 4.25).

Table 4.25 Prevalence of subjective symptoms of the side-effects of caffeine experienced in the caffeine and placebo groups

	Caffeine & placebo combined	Caffeine	Placebo	Caffeine vs. Placebo
	% (N)	% (N)	% (N)	p-value
All	N = 52	N_c = 26	N_p = 26	
Nervousness	54% (28)	38% (10)	54% (14)	$p = 0.26$
Shakiness	27% (14)	42% ^a (11)	12% ^b (3)	$p = 0.00^*$
Anxiety	23% (12)	23% (6)	23% (6)	$p = 1.00$
Heart palpitations	23% (12)	38% ^a (10)	8% ^b (2)	$p = 0.01^*$
Flushing	10% (5)	15% (4)	4% (1)	$p = 0.15$
GIT disturbances	23% (12)	38% ^a (10)	8% ^b (2)	$p = 0.01^*$
Headaches	17% (9)	12% (3)	23% (6)	$p = 0.27$
Male	N_m = 28	N_{cm} = 14	N_{pm} = 14	
Nervousness	32% (9)	29% (4)	36% (5)	$p = 0.69$
Shakiness	25% (7)	43% (6)	7% (1)	$p = 0.23$
Anxiety	18% (5)	21% (3)	14% (2)	$p = 0.62$
Heart palpitations	21% (6)	36% (5)	7% (1)	$p = 0.06$
Flushing	11% (3)	14% (2)	7% (1)	$p = 0.54$
GIT disturbances	14% (4)	29% ^a (4)	0% ^b (0)	$p = 0.01^*$
Headaches	18% (5)	14% (2)	21% (3)	$p = 0.62$
Females	N_f = 24	N_{cf} = 12	N_{pf} = 12	
Nervousness	63% (15)	50% (6)	75% (9)	$p = 0.20$
Shakiness	29% (7)	42% ^a (5)	17% ^b (2)	$p = 0.05^*$
Anxiety	29% (7)	25% (3)	33% (4)	$p = 0.65$
Heart palpitations	25% (6)	42% ^a (5)	8% ^b (1)	$p = 0.05^*$
Flushing	8% (2)	17% (2)	0% (0)	$p = 0.09$
GIT disturbances	33% (8)	50% (6)	17% (2)	$p = 0.08$
Headaches	17% (4)	8% (1)	25% (3)	$p = 0.26$

^a differed significantly from ^b ($p < 0.05$)

N = total sample; N_m = total male sample; N_f = total female sample; N_c = total caffeine; N_{cm} = total caffeine male; N_{cf} = total caffeine female; N_p = total placebo; N_{pm} = total placebo male; N_{pf} = total placebo female

4.5.8.1 Influence of the symptoms of the side-effects of caffeine supplementation on the overall time to complete the triathlon

The researcher did a factorial ANOVA with VEPAC of the overall time to complete the triathlon with factors group and caffeine and then gender and caffeine with respondents

nested firstly in group and then nested in gender. To investigate the influence of the different symptoms of the side-effects of caffeine, these variables were one by one entered as covariates in a similar analysis of covariance with factors (group and caffeine and then gender and caffeine) as described above.

Of all the side effects considered, heart palpitations were the only covariate which significantly affected the overall time to complete the triathlon ($p = 0.03^*$) (Figure 4.27). In the caffeine and placebo groups, irrespective of gender, the prevalence of heart palpitations led to a slower time to complete the triathlon.

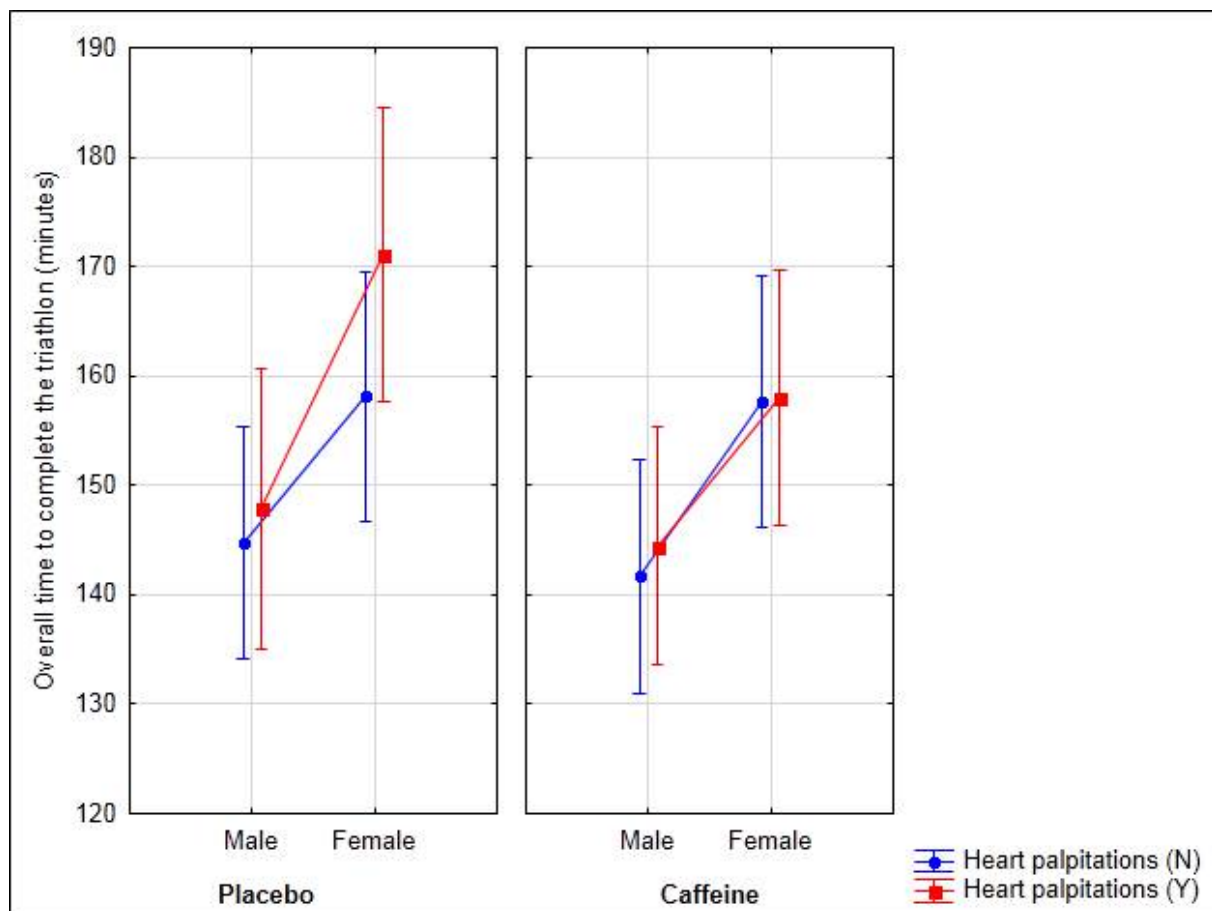


Figure 4.27 Overall time to complete the triathlon according to the prevalence of heart palpitations experienced (Time effect: $p = 0.03^*$).

4.5.9 Hydration status and changes in plasma volume (serum albumin)

The researcher performed a RMANOVA with VEPAC on serum albumin levels with factors group, caffeine and stage (baseline, during transition (cycle → run) and at the finish line);

and then gender, caffeine and stage; with respondents nested firstly in group ($p = 0.26$) and then in gender ($p = 0.12$) (Table 4.26).

Table 4.26 Serum albumin levels in the caffeine and placebo groups

	All	Caffeine	Placebo	Caffeine vs. Placebo
	Mean±SD	Mean±SD	Mean±SD	p-value
All	$N = 52$	$N_c = 26$	$N_p = 26$	
Baseline (g/l)	48.6±2.4	48.6±2.2	48.6±2.6	$p = 1.00$
Transition (cycle-run) (g/l)	49.9±2.6	50.0±2.6	49.8±2.7	$p = 0.72$
Finish line (g/l)	48.7±2.5	48.8±2.2	48.7±2.7	$p = 0.72$
Male	$N_m = 28$	$N_{cm} = 14$	$N_{pm} = 14$	
Baseline (g/l)	48.9±1.9	48.5±1.7	49.4±2.0	$p = 0.12$
Transition (cycle-run) (g/l)	51.1±2.2	51.2±2.0	51.0±2.4	$p = 0.70$
Finish line (g/l)	49.2±2.5	49.4±2.3	49.1±2.9	$p = 0.61$
Female	$N_f = 24$	$N_{cf} = 12$	$N_{pf} = 12$	
Baseline (g/l)	48.3±2.8	48.8±2.7	47.8±2.9	$p = 0.10$
Transition (cycle-run) (g/l)	48.5±2.4	48.6±2.5	48.5±2.3	$p = 0.90$
Finish line (g/l)	48.2±2.3	48.2±2.0	48.2±2.6	$p = 1.00$

N = total sample; N_m = total male sample; N_f = total female sample; N_c = total caffeine; N_{cm} = total caffeine male; N_{cf} = total caffeine female; N_p = total placebo; N_{pm} = total placebo male; N_{pf} = total placebo female

Serum albumin levels measured during transition (cycle → run) increased significantly from serum albumin levels measured at baseline ($p = 0.00^*$). Thereafter, serum albumin levels measured at the finish line decreased significantly from serum albumin levels measured during transition (cycle → run) ($p = 0.00^*$). The serum albumin levels measured at baseline did not differ significantly from the serum albumin levels measured at the finish line ($p = 0.70$) in the whole group.

The difference observed between the various stages of the triathlon was not due to caffeine supplementation in males or females ($p = 0.12$). Although there were no overall observed effects between gender, caffeine supplementation and serum albumin levels (at all three time points) ($p = 0.12$), there was a significant difference in serum albumin levels measured at the three time points for males and females ($p = 0.00^*$), with males having consistently higher values than females (Figure 4.28).

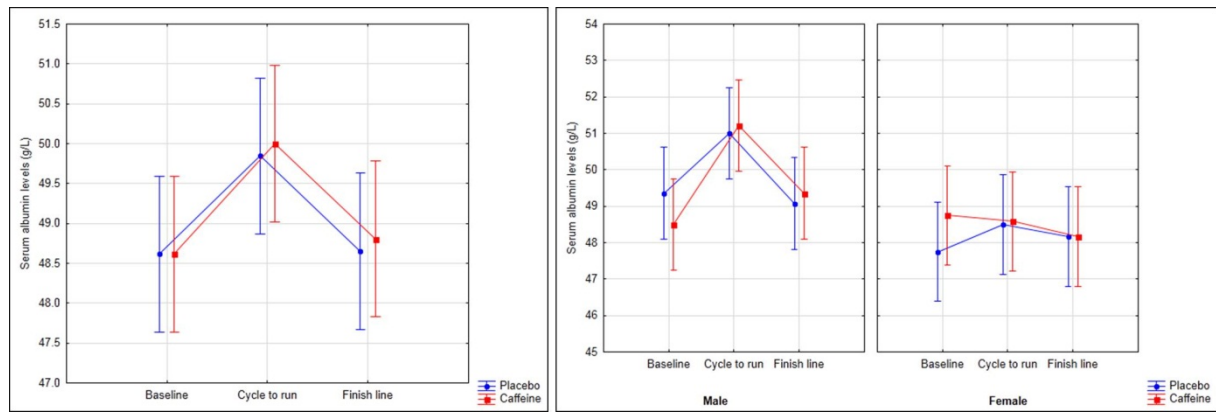


Figure 4.28 Serum albumin levels measured at baseline, during transition (cycle → run) and at the finish line in the caffeine and placebo groups (Supplementation effect: $p = 0.96$) and according to gender (Gender effect: $p = 0.00^*$)

CHAPTER 5: DISCUSSION

This research study provides a comprehensive assessment of the performance enhancing or ergogenic effect of caffeine supplementation during a real life triathlon competition, an evaluation of several parameters that could in part explain why caffeine supplementation is ergogenic during endurance exercise and it describes in detail factors influencing the ergogenicity of caffeine supplementation as well as factors influencing triathlon performance. The researcher demonstrated that double-blind, randomized, crossover, controlled, clinical field trials, and not only laboratory experiments, are necessary to establish the real effect of caffeine supplementation on triathlon performance. It was shown that caffeine supplementation during an Olympic-distance triathlon has a performance enhancing effect on male and female provincial level triathletes in the Western Cape, South Africa. However, this effect was not as pronounced as seen in previous laboratory trials and the effect was not significantly related to a reduction in rating of perceived exertion or positive changes in mood state. Further results discussed in this chapter indicated that caffeine supplementation acts mainly as an ergogenic aid through its affect on the central as well as autonomic nervous system. Factors such as lifestyle, gender and genetics may influence the ergogenicity of caffeine supplementation although this remains to be substantiated in larger cohorts. General health, dietary intake, body composition and bone mineral density, side effects of caffeine withdrawal and acute caffeine supplementation and hydration status may influence Olympic-distance triathlon performance.

5.1 Ergogenic effect of caffeine supplementation

The present study investigated the effect of 6 mg/kg BW microencapsulated caffeine (70% caffeine concentration,[©] 2005-2009 Maxx Performance Inc.) on triathlon performance.

The reference range for plasma caffeine concentrations is 5-25 mg/l. In the current study, the baseline plasma caffeine levels were < 1 mg/l, indicating that the athletes adhered to the research protocol by abstaining from caffeine supplementation.

The peak plasma caffeine concentration to have an ergogenic effect (8-10 mg/l) for doses of 5-8 mg/kg body weight is typically reached in the blood after 15-120 minutes (57), and therefore the caffeine supplementation was given to the subjects in the present study 45-60 minutes prior to the start of the triathlon. Peak plasma caffeine concentrations (7-10 mg/l) were reached in the caffeine group during transition (cycle → run) and at the finish line. The supplementation was therefore effective in elevating plasma caffeine concentrations to levels which have been shown to be ergogenic in the literature.

5.1.1 Triathlon performance (time to complete)

Most studies evaluating the effect of caffeine supplementation on endurance performance showed an improved performance when using a TTE protocol, followed by a TT or only a TTE protocol (13-16, 19-22, 25-30, 41, 45, 75, 82, 83, 85, 100-105, 107, 111, 114, 115, 209).

No improvement in performance was found in studies evaluating the effect of caffeine supplementation on endurance exercise, during various extreme environmental and exercise conditions (37, 53, 76, 86, 90, 92, 98, 106, 109, 110, 115).

In the current study, the researcher found statistically significant differences in the time to complete the swim and the overall time to complete the triathlon in the caffeine group (a 3.7% and 1.3% reduction respectively) when considering the group as a whole. The time to complete all the other components, including cycle time and run time, was not reduced to a statistically significant extent, although there was a 1.1% and 0.7% decrease in the time to complete the cycle and run, respectively. When considering the times in a gender-specific manner, the overall time to complete the triathlon was significantly reduced (1.7%) in the male group receiving caffeine. Although the time to complete the swim improved by 4.5%, the cycle by 1.8% and the run by 1.1%, these decreases in time were also not statistically significant. The females also showed a percentage reduction in the swim (2.8%), cycle (0.5%) and run (0.4%) times, as well as the overall time to complete the triathlon (0.9%), but this too was not statistically significant.

The fact that the abovementioned decreases in time were not found to be statistically significant may be due to the small sample size used. However, studies (Table 2.1) that showed a significant improvement used smaller sample sizes, suggesting rather that this non-significant effect was rather due to the effect of competing in a real-life triathlon than only the sample size employed. A triathlon encompasses three disciplines and cannot be compared to studies completed solely on swimming, cycling and running. Furthermore, a field study cannot readily be compared to studies conducted in a laboratory environment.

It is evident from the results, that caffeine supplementation taken 45-60 minutes before an Olympic-distance triathlon, exerted the greatest effect on performance in the swimming section of the triathlon, as well as on the overall time to complete the triathlon, in the whole subject group.

The effect of caffeine supplementation on swim performance in the present study is interesting. Swimming is not regularly chosen as an exercise protocol when evaluating the ergogenic effect of caffeine (Table 2.1). A reason for this may be that the intensity of exercise cannot be regulated in the same manner as cycling or running and pacing strategies may differ whilst swimming (15). The few studies that have examined the effect of caffeine supplementation on swim performance either evaluated the effect during short distance swimming (100 m or less) (210, 211) or the combination of caffeine and sodium bicarbonate on repeated short distance (200 m) swim performance (212). The only study to the author's knowledge that evaluated the effect of caffeine supplementation on 1 500 m swim performance was conducted in 1995 (15). The authors concluded that supplementation with 6 mg/kg caffeine, two and a half hours prior to the 1 500 m swim significantly reduced the time to complete the 1 500 m swim. The authors suggested that this ergogenic effect may have been due to altered electrolyte balance and improved glucose availability (15). In the present study, the researchers also found the most profound decrease in the time to complete the swim section of the triathlon. The present study did not evaluate the same mechanisms in terms of electrolyte balance and glucose availability, but found that caffeine supplementation continually increased plasma caffeine levels from baseline, with the highest values recorded at the finish line. This would suggest that caffeine supplementation would have a beneficial effect, even before peak plasma caffeine levels have been reached. Rating of perceived exertion was also lower in the caffeine compared to placebo group (although not statistically significant) and lowest during the transition from the swim → cycle. This suggests that caffeine has a beneficial effect before maximal exertion or fatigue is reached, such as experienced at the end of the cycle or run leg of the triathlon. The importance of an increased swim performance during a triathlon is of paramount importance, especially when applying the results to elite triathletes who race in draft-legal triathlon races. The evaluation of caffeine supplementation on swim performance warrants further research.

The practical application of these results for triathletes is that caffeine supplementation decreases time to complete an Olympic-distance triathlon in the field setting. Even though we had a relative small sample size, this effect was evident in both males and females. The greatest effect was seen on the overall time to complete the triathlon, as well as the swim section of the triathlon. It was the shortest discipline of the three and also the first. Although not always statistically significant, the percentage decrease in time to complete the various sections (swim, cycle, and run) of the triathlon is important to note. One to two minutes decreased time to swim can make a significant practical/clinical difference in triathlon performance. This would be even more important in races where the cycling section of the triathlon is draft legal and making the lead pack from the swim to the cycle is of critical

importance. Although the present study was not draft legal, this is of practical value for draft legal races.

5.1.2 Rating of perceived exertion (RPE)

RPE is one of the well-studied outcomes when evaluating the literature on the effect of caffeine supplementation. Various studies have found no change in RPE with caffeine supplementation (75, 77, 83, 85, 87, 102, 116). However, most studies have shown decreased RPE with caffeine supplementation (14, 15, 22, 27, 29, 31, 32, 37, 45, 81, 91, 92, 96, 97, 99, 100, 104, 109, 110, 112, 117).

In all studies evaluating the mechanism of action of the ergogenic effect of caffeine supplementation, or the relationship of caffeine to improved exercise performance, the enhanced effect of caffeine on a person's perceptual responses remains constant (31). In a meta-analysis conducted by Doherty and Smith *et al.* (2005), it was concluded that there was a 6% reduction in RPE during constant load exercise, but no difference in RPE values after exhaustive exercise. The author concluded that this was a normal physiological response, as exercise would be stopped when the individual was exhausted or at the end of exercise. This is the main reason for most studies reporting an improvement in RPE when determining time to exhaustion (31).

The abovementioned meta-analysis, along with another meta-analysis by Doherty and Smith *et al.* (2004), concluded an 11% reduction in RPE and improved exercise performance following caffeine ingestion (28, 31). The decrease in the RPE during endurance exercise enables athletes to manage fatigue, which allows the athletes to complete longer TTE protocols. The difference in perceptual response allows subjects to recruit and use more motor units and improve power output, which can also be beneficial for time trial performance (31). However, in the current study, none of the RPE measures influenced the time to complete any single component of the triathlon or the overall time to complete the triathlon.

The meta-analysis conducted by Doherty *et al.* (2005) concluded that the effect of caffeine on RPE was present, irrespective of caffeine withdrawal, or the timing or dosage of caffeine supplementation, and that there was a more pronounced decrease in RPE with advanced levels of fitness (31).

The current study showed no statistically significant differences in the RPE measured at baseline, transition (swim → cycle), transition (cycle → run) and at the finish line between the caffeine and placebo groups, but there was a clear trend towards lower RPE values in the caffeine group. This concurs with research conducted by Tarnopolsky *et al.* (1989) and Trice *et al.* (1995), who found a 16% and 6% reduction, respectively, in RPE, although these results were also not statistically significant (22, 112).

The reason for the abovementioned studies and others showing little or no improvement in RPE can, according to Doherty *et al.* (2005) be attributed to the small sample sizes used (31). However, in the current study, the sample size was the same, if not greater, than the sample sizes used in most of these studies. A further explanation for the lack of statistical significance can be that the current study was a field study, not a laboratory-based study. In field studies, various additional physiological factors may be involved, and these can affect perceptual responses and levels of fatigue. Moreover, the current study involved studying the effect of caffeine supplementation during and after a triathlon, which has not been studied previously. Physiological responses during and after competing in a triathlon are also different from responses during and after only cycling or running and can have an effect on the athlete's perceptual response to exercise. It is also important that during a time trial event, RPE cannot be interpreted in the same manner as during a time to exhaustion or constant exercise at submaximal intensity. During a time trial, even though the athlete is less fatigued from the exhaustive exercise due to caffeine supplementation, the athlete will push him/herself harder and RPE will remain the same. Performance time is therefore a greater indicator in a time trial event than measures of RPE. There is, nevertheless, a clear trend in most studies, including the current study, suggesting that caffeine supplementation reduces RPE, although, the percentage decrease in outcomes measured, is not always statistically significant.

5.1.3 Mood state

Subjects also completed the POMS questionnaire at baseline and again at the finish line of the triathlons, in order to determine the effect of caffeine supplementation on the mood state of the subjects. It has been reported that caffeine significantly improves mood (195), although this has not been measured consistently with the POMS questionnaire. Caffeine has been found to be positively associated with mood, contributing to individuals feeling more energetic, imaginative, efficient and motivated to work and socialize (57).

Caffeine supplementation in the current trial had no effect on the total or differential POMS scores in all subjects, and the male and female groups. This is in contrast to other studies that examined the effect of caffeine-containing energy capsules on mood and psychomotor performance in fatigued individuals. Childs *et al.* (2008), who also used a POMS questionnaire, found that the ingestion of a caffeine-containing food supplement improved the subjective state and cognitive performance of fatigued individuals, and also that caffeine had a stimulant-like effect in improving mood and reaction times (213). Possible reasons why caffeine supplementation did not improve POMS scores could be that the baseline POMS questionnaire was completed before caffeine supplementation and that supplementation could not have had an effect and that the POMS score at the finish line was after the triathlon, which is exhaustive exercise. The effect of caffeine supplementation on the POMS score may not have been big enough to detect. It could also be that in the present study, the sample size was too small to detect changes in the POMS score due to caffeine supplementation.

The mood state of the athletes was not influenced by caffeine supplementation. We can therefore conclude that either the athletes were not sufficiently mentally fatigued for caffeine to have a measurable beneficial effect or the mechanism by which caffeine elicits its ergogenic effect is not clear from the current study's results, as mood state was unchanged regardless of caffeine supplementation.

It is clear from the present study that caffeine supplementation significantly improves the time to complete an Olympic-distance triathlon. However, the mechanism by which caffeine elicits its ergogenic effect is not clear from the current study's results as rating of perceived exertion and mood state was statistically unchanged due to supplementation with caffeine. The researcher did however observe a general trend toward lower RPE values in the caffeine when compared to the placebo group. If these results can be substantiated in a larger cohort and significantly reduced RPE values observed, the effect of caffeine supplementation can be attributed to one of two suggested mechanisms of action. First, the antagonism of adenosine receptors, which leads to a reduction of pain, improving arousal and motivation and thus reducing pain and RPE or to the stimulation of the HPA-axis, which leads to the release of β -endorphins and cortisol, both of which has been shown to decrease RPE .

5.2 Parameters that could in part explain the ergogenic effect of caffeine supplementation

5.2.1 Endocrine-stress response

5.2.1.1 Serum cortisol levels

In the current study, all the measured serum cortisol values (at baseline, 1 hour before the triathlon and again at the finish line) were within the recommended reference range (140-700 nmol/l). Cortisol levels follow a circadian rhythm in the body, levels peak at about 8 a.m. and then decrease during the day. The serum cortisol level measured at the finish line was significantly higher in the caffeine group, compared to the placebo group. This was particularly evident in the male group.

As discussed in the literature overview, cortisol levels are increased independently due to caffeine, the mental stress of competing in a race environment and the exertion of exercising at the high intensity required during a triathlon (140). Several studies have found increased cortisol levels after marathon running (136, 142-146). Balthazar *et al.* (2012) found that salivary cortisol were greater on the day of a short triathlon, compared to a rest day, in eight male triathletes, and that exercise performance was positively associated with salivary cortisol concentration in the early morning on race day. The authors concluded that early morning salivary cortisol levels could be used to predict performance during a triathlon (214).

Exercise is also a metabolic stressor and the response of cortisol to stress varies widely between individuals (148), between genders and between various durations of exercise (136, 145, 147). The elevated cortisol levels seen after exercise may be a largely metabolic effect, which lead to increased gluconeogenesis and lipolysis. Our results are therefore in agreement with the sports literature in this context.

There was a difference between male and female cortisol levels, with males reaching a significantly higher serum cortisol level at the finish line, compared to females. This is also supported by previous studies that have shown that in females, caffeine supplementation has a smaller effect on cortisol levels than in males. In the current study, differences between cortisol levels in males and females were observed, irrespective of whether caffeine supplementation was administered or not. Cortisol levels in males were increased after exercise, whereas these levels decreased in the female group. The serum cortisol levels measured at baseline also influenced the overall time to complete the triathlon, with faster times being observed in males and slower times in females.

Studies found that the cortisol response to mental stress is less pronounced in females compared to males (140, 215). This author also found that 30 minutes of steady state exercise on a cycle ergometer had no effect on cortisol levels, but when caffeine was given before exercise, serum cortisol levels were elevated in both males and females (140). Caffeine has been shown to elevate cortisol levels at rest and after exercise (51, 131, 140, 216). It therefore appears that the release of cortisol in response to stress or caffeine is dependent on the type of stressor, as well as gender. It was also concluded that caffeine increases cortisol secretion by stimulating the CNS, especially in males, but in females it may interact with peripheral metabolic mechanisms (140).

The augmented increase in cortisol levels seen after exercise with caffeine supplementation suggests that one of the main mechanism of action of caffeine having an ergogenic effect is the indirect stimulation of the HPA-axis and the effect on the autonomic nervous system (in addition to the effect on the CNS). This leads to cortisol levels being elevated above that would occur after prolonged or endurance exercise. The increased cortisol can positively affect cognitive performance and mood, thereby decreasing the perception of pain and improving sport performance.

5.2.1.2 DHEAs levels

In the present study, there was a significant difference between baseline DHEAs levels in the caffeine and placebo groups. This was, however not due to caffeine supplementation as this was given after the baseline blood samples were taken. DHEAs levels were consistently within the reference ranges (i.e. 0.95-11.7 umol/l for males and 2.17-15.2 umol/l for females). There were significant increases between baseline and finish line DHEAs levels in all subjects, and the male and female groups, irrespective of whether or not caffeine supplementation was given. There were no statistically significant differences between DHEAs levels in males and females, although there were lower baseline and finish line levels observed in females compared to males. According to the literature and as discussed in the literature review, DHEAs levels increase during endurance exercise (136, 150). It is also known that women, who regularly enjoy endurance exercise, such as the females in our study group, have decreased resting DHEAs levels as a result of decreased ACTH following an endurance training program (135). When introducing DHEAs levels as a covariate to triathlon performance, no significant influence was found.

5.2.1.3 Prolactin levels

Prolactin levels measured at baseline and at the finish line, also consistently fell within the recommended reference ranges (2.6-13.1 ug/l for males and 3.3-26.7 ug/l for females). In the caffeine and placebo groups, baseline and finish line serum prolactin levels increased significantly in all subjects, and the male and female groups. There were no differences observed between the caffeine and placebo groups. There was also no difference observed between the male and female group, although the average baseline and finish line values were slightly higher in the female group. The finish line levels in the female group also increased more markedly from baseline than in the male group (51% increase for females, compared to 41% increase for males). Karkoulas *et al.* (2008) reported an increase in prolactin levels due to physical stress, such as endurance exercise (136). This is particularly evident in female athletes following endurance exercise as described by Enea *et al.* (2011) (135).

The introduction of serum prolactin as a covariate to influence triathlon performance showed no effect on the overall time to complete the triathlon. It has been hypothesized that prolactin secretion could be reduced during an exercise trial following caffeine ingestion as prolactin is under control of the central serotonergic system. Without caffeine supplementation, prolactin increases serotonin synthesis and decreases dopamine synthesis. Caffeine has the opposite effect and therefore decreased levels of prolactin could be observed. However, this was not observed in the present study as levels between caffeine and placebo groups remained the same. This may suggest that the physical stress of strenuous exercise in near real life conditions increase prolactin (with increased cortisol levels) and caffeine decreases prolactin, therefore, the net effect is no/little change in prolactin levels. However this remains to be substantiated by further research.

5.2.1.4 Testosterone

In the present study, all the testosterone values fell within the reference range (9.9-27.8 nm/l for males and 0.22-2.0 nm/l for females). In the caffeine and placebo groups, significantly lower values were obtained at the finish line, compared to baseline values in the whole group and in the male group. This is in keeping with studies reporting decreased testosterone levels during and after endurance exercise (136).

As expected, however there was a significant difference in the testosterone levels between males and females. Males had significantly higher baseline and finish line values, with the values at the finish line significantly lower than the value measured at baseline.

In the female group, there was no difference between baseline and finish line values. Enea *et al.* (2011) found that females had increased testosterone levels during and after endurance exercise (135). This was not supported by the current study, in which there was no statistically significant difference in the testosterone levels of the female group between the baseline and the finish line, although a slight decrease was noted in testosterone levels at the finish line. This could in part be due to triathlon being more intensive exercise than those described by Enea *et al.* (2011) (135).

There were also no differences in values between the caffeine and placebo groups. During resistance training, caffeine have been found to increase testosterone levels after training, although the effect may be diminished by an increased release of cortisol due to physical activity and caffeine as a metabolic stressor (152). The introduction of testosterone as a covariate had no influence on triathlon performance.

The increased DHEAs levels are consistent with literature indicating that prolonged exercise can increase DHEAs and consequently decrease testosterone levels, the observed effect in the present study. In addition, in the present study it was observed that testosterone levels decreased and cortisol levels increased. Therefore, the effect of caffeine supplementation on cortisol is greater than the effect thereof on testosterone levels, suggesting the stimulation of the HPA-axis being a primary mechanism of action of caffeine supplementation (in addition to the effect on the CNS). If the primary effect was only on the CNS, testosterone levels would have increased irrespective of increased cortisol levels. However, due to stimulation of the HPA-axis, cortisol levels increased and negated the effect of caffeine supplementation on testosterone levels, suggesting that the latter is not the only mechanism of action.

In conclusion, caffeine did not seem to have major effects on the endocrine-stress response to exercise, except for cortisol, which increased beyond that of the effect observed from endurance exercise in the caffeine, compared to placebo group. Other markers displayed gender differences as expected, but no differences were observed in the caffeine and placebo groups. Exercise stress resulted in increased levels of cortisol, DHEAs and prolactin and decreased testosterone levels. Furthermore, caffeine supplementation exacerbated this response by further elevating levels of cortisol, suggesting a combined effect of the central and autonomic nervous system on the ergogenicity of caffeine supplementation.

5.2.2 Oxidative-stress

Fifty to sixty per cent of circulating leukocytes are neutrophils, which are known as the first line of defence against infectious agents. Neutrophils release reactive oxygen species (ROS) and antimicrobial enzymes (133). Strenuous exercise, for example a triathlon race, can promote oxidative stress. It was therefore important to measure the total and differential leukocyte count to assess whether caffeine supplementation had an effect on this first line of defence after exercise.

5.2.2.1 White blood cell (WBC) count

White blood cells, also known as leukocytes, are mainly responsible for defending the body against foreign entities, such as bacteria, parasites and viruses. White blood cells can be divided further into the differential white cell count, which includes lymphocytes, monocytes, neutrophils, eosinophils and basophils. Lymphocytes are also termed immunocytes and are primarily responsible for the body's immune response against infections. Monocytes leave the circulation, enter tissues and develop into macrophages. Macrophages, monocytes and neutrophils are known as phagocytes, due to their ability to engulf and ingest foreign particles and thereby destroy these. Neutrophils, eosinophils and basophils form part of the granulocytes, as the cytoplasmic content of these cells gives it a granular appearance (5).

Both the absolute number of WBC and the relative proportions of the different types of WBC in the circulation will change when the body is called to fight foreign entities (5).

The total WBC count should not be interpreted in isolation. The absolute counts of individual cell types comprising the WBC count should also be taken into consideration (217). The normal reference range for the total WBC count is $4 - 11 \times 10^9/l$ for males and females. In the current study, all the WBC count values (all subjects, males and females) increased from baseline to the finish line. All the baseline values fell within the recommended reference ranges, but all values measured at the finish line exceeded $11 \times 10^9/l$. This increase was significantly higher in the caffeine group in all subjects and in the male group. Although not statistically significant, there was a trend towards higher WBC count values at the finish line in the female group as well. In general, the males also had significantly higher WBC values at the finish line, compared to the female group. This increased WBC count observed at the finish line and due to caffeine supplementation could be due to exercise and caffeine independently being stressors. The WBC count increases when acute inflammation or infection is present, as well as in dehydration states. This increased leukocytosis is observed primarily due to increased neutrophils and lymphocytes and is a result of the effect of

caffeine on the central nervous system or as a result of the effect of caffeine on the autonomic nervous system (133), whereby caffeine increases cortisol release after exercise (as seen in the present study), the increased cortisol leads to increased neutrophils being recruited from bone marrow to the circulation, resulting in leukocytosis and neutrophilia (133).

Studies on resistance exercise have found that, with caffeine supplementation, leukocyte counts did not increase any more than that resulting from exercise alone (218). Walker *et al.* (2007) also found that caffeine supplementation had no effect on leukocyte count following time trial cycling performance (133).

5.2.2.2 Neutrophils

Neutrophils comprise approximately 70% of the total WBC count. Neutropenia, a low neutrophil count, can arise from viral infections, autoimmune diseases, idiopathic causes and drug use. It is important to note that non-steroidal anti-inflammatory drugs, regularly taken by many athletes, can induce a mild state of neutropenia. In contrast, neutrophilia is a state of a high neutrophil count and usually increases within one hour of tissue injury. It is the hallmark of acute inflammation and any stressor, including bacterial infections, smoking, heavy exercise, nervousness, medication and pregnancy can increase neutrophil counts (217).

The normal reference range for the absolute neutrophil count is $2-8 \times 10^9/l$. Results from the present study indicate increased neutrophil counts for all subjects, and the male and female groups at the finish line of the triathlon. Baseline values all fell within the normal range. There was a significant increase in the neutrophil count observed in all subjects, and the male group at the finish line in the caffeine group, compared to the placebo group. Although this was not statistically significant, the females, when receiving caffeine, also had higher neutrophil counts when compared to the placebo group at the finish line. There was also a significant difference in the neutrophil count values measured between males and females. When introducing the neutrophil count as a covariate to determine the influence of this on triathlon performance, no significant correlation was found.

Apart from the absolute neutrophil values, the relative proportions of the different types of WBC in the circulation will change in response to infection or inflammation (5). It is important to consider the relative neutrophil count in the current study; all of the values for the baseline relative neutrophil count fell within the reference range of 40-75% of WBC count. All relative

neutrophil counts at the finish line in all subjects, and the male and female groups were slightly higher than 75% of the WBC count. There was a significant increase in the relative neutrophil count from baseline to the finish line in all subjects, and the male and female groups; however, this change was not due to caffeine supplementation. There was a significant difference in the relative neutrophil count measured at baseline and at the finish line between the male and female groups, with females exhibiting consistently higher values than males. The relative neutrophil count also had no significant influence on the overall time to complete the triathlon.

Neutrophils act by releasing ROS and antimicrobial enzymes to form part of the body's first line of defence against infectious agents. This response is usually decreased after intense endurance exercise (219). In the present study, caffeine increased neutrophil counts above that observed due to endurance exercise. This is not in keeping with other studies, such as the one by Walker *et al.* (2006) on 90 minute cycling at 70% VO_2 max, followed by a time trial, which found that caffeine supplementation had no effect on neutrophil counts (132). The difference in these results may be due to the exercise protocol in the present study being a triathlon, and a race, which implies completing the set amount of work in the fastest time possible (i.e. not steady state exercise with a time trial at the end). The extra neutrophil counts found in the current study may suggest higher oxidative stress, although other, more accurate markers of oxidative stress needed to be studied to confirm this.

The increase in neutrophils also suggests that the primary mechanism of action of caffeine is antagonism of the adenosine receptors (133). However, as increased cortisol levels were also observed, leukocytosis and neutrophilia could have resulted due to stimulation of the autonomic nervous system as increased cortisol increases recruitment of neutrophils from bone marrow to the circulation (133). A single study on soccer players reported neutrophilia after increased muscle stress resulting from caffeine supplementation and concluded that this may increase the risk of muscle damage in athletes (52). This avenue remains to be further elucidated since it was not the focus of this study.

5.2.2.3 Lymphocytes

Lymphocytes normally represent between 20-40% of the WBC count. Lymphocytopenia (low lymphocyte counts) can be associated with acquired conditions, such as infectious diseases (such as AIDS, viral hepatitis, tuberculosis and typhoid fever), autoimmune disorders, steroid therapy, blood diseases (such as Hodgkin's disease and aplastic anemia) and radiation or chemotherapy or due to rare inherited causes (such as Wiskott-Aldrich syndrome, severe

combined immunodeficiency syndrome and ataxiatelangiectasia). In contrast, lymphocytosis (high lymphocyte counts) can be present due to acute viral or bacterial infections, smoking, autoimmune response or acute stress (217).

In the current study, the absolute lymphocyte count values in all subjects, and the male and female groups, fell within the recommended reference range of $1.0 - 4.0 \times 10^9/l$. There was a significant increase in absolute lymphocyte count values between the baseline and the finish line in all subjects when receiving caffeine supplementation as well as in the male subjects receiving caffeine. However, there was no significant difference in the lymphocyte counts between the baseline and finish line in the female group receiving caffeine. None of the measurements in the placebo group were statistically significantly different from the baseline to the finish line. The increased absolute lymphocyte count measured at the finish line in all subjects and the male group, was due to caffeine supplementation.

There was a trend towards higher lymphocyte count values at the finish line in the female group receiving caffeine, compared to the placebo group. There were no differences between lymphocyte counts measured for males and females. The lymphocyte count also did not influence the overall time to complete the triathlon.

The relative proportion of lymphocytes as part of the WBC count should be between 20-45%. The baseline relative lymphocyte count values fell within this recommended range, but finish line values for all subjects, and the male and female groups were below 20% of WBC count. The finish line values were also significantly lower than the baseline values, although this difference was not due to caffeine supplementation. There was a significant difference in the relative lymphocyte count between males and females, with males having consistently higher values than females, and the reduction in the relative count from baseline to finish line was more pronounced in males than in females.

Our results, which showed an increase in the absolute lymphocyte count at the finish line (which was not an effect of altered hydration status), are in contrast to those reported by Walker *et al.* (2006), who found that after steady state and time trial cycling performance, the lymphocyte count was significantly lower in the caffeine group, compared to the placebo group (132). This may be due to the acute stress of competing in a triathlon race, compared to steady state exercise in a controlled laboratory environment. However, in keeping with the results from the present study, Bishop *et al.* 2005 also reported increased lymphocytes following a strenuous cycling protocol (220). The observed increase in lymphocyte counts can also be attributed to the effect of caffeine on the central nervous system, as seen with

increased neutrophil counts in the present study. The total increase in leukocytes may also increase muscle stress and the risk of muscle damage in athletes (52).

5.2.2.4 Other WBC counts

Monocytes, eosinophils and basophils are discussed below. To the researcher's knowledge, limited studies exist on these parameters with regard to endurance exercise and caffeine supplementation. This study provides new information on the effect of exercise and caffeine on these parameters of the WBC count.

Monocytes make up 3 – 8% of the WBC count. Monocytes typically stay in the bloodstream for 1-3 days, and are then taken up by tissues and converted to macrophages with a specific function as part of the immune system and phagocytosis. Monocytopenia (low monocyte count) can be due to acute infections, stress, treatment with glucocorticoids, aplastic anaemia, hairy cell leukaemia, acute myeloid leukaemia, treatment with myelotoxic drugs and genetic syndromes (221). Monocytosis (high monocyte count) can indicate chronic infection or inflammation, especially when elevated with other components of the WBC count (217).

All the monocyte values in the present study fell within the recommended reference range of $0.0 - 1.0 \times 10^9/l$. There was a significant increase in the values measured at the finish line, compared to baseline values in all subjects, and the male and female groups. This increase was only due to caffeine supplementation in the entire group and this effect was not seen when evaluating genders apart. There were also no significant difference between monocyte values between males and females. The monocyte count did not have an influence on the overall time to complete the triathlon.

The reference range for the relative contribution of monocytes to the WBC count is 2-10%. In the present study, all groups had relative counts within this range. However, in contrast to the absolute monocyte count, which increased from baseline to the finish line, the relative monocyte count decreased in all subjects, and the male and female groups. This decrease was not due to caffeine supplementation and did not influence the overall time to complete the triathlon. There were significant differences between the relative monocyte counts for males and females, with males having higher relative counts at baseline and lower relative counts at the finish line.

Eosinophils comprise 1-6% of the total WBC count. Eosinopenia (low eosinophil count) is difficult to determine as the values are normally very low. This is therefore not of clinical importance. Eosinophilia (high eosinophil count) can be present in allergic conditions, such as asthma or hay fever, as well as in parasitic infections (217).

All eosinophil values fell within the recommended range of $0.0-0.5 \times 10^9/l$ in the present study. There was a decrease in values measured at the finish line, compared to baseline values in all subjects, and the male and female groups. However, this decrease was not due to caffeine supplementation. There were also no significant differences between values obtained for males and females and the eosinophil count had no effect on the overall time to complete the triathlon.

The percentage contribution of eosinophils to the total WBC count decreased from the baseline to the finish line during the present study. Baseline measurements were well within the recommended 1-6% of WBC count, but the finish line measurements were consistently below 1% of the WBC count for all subjects, and the male and female groups. This difference was not due to caffeine supplementation and is not clinically significant. Relative eosinophil counts did not differ significantly between males and females, and did not influence the overall time to complete the triathlon.

Basophils are the least common component of the differential WBC count, constituting 0-1% of this. The functions of basophils are poorly understood, but these cells are known to play a role in phagocytosis and in producing histamine, a mediator responsible for allergy symptoms and inflammation. Basopenia (low basophil count) is also difficult to detect, due to the already low contribution of these cells to the total WBC count, and is not clinically significant. Basophilia (high basophil count) only occurs in rare circumstances (217).

The absolute basophil count fell within the recommended reference range of $0.0-0.2 \times 10^9/l$ in all subjects, and the male and female groups, when measured at baseline and at the finish line. There was a significant increase observed from baseline to finish line values in these groups. However, this increase in the absolute basophil count was not due to caffeine supplementation and did not differ between males and females. The absolute basophil count did not influence the overall time to complete the triathlon.

In contrast to the absolute basophil count mentioned above, there was a decrease in the relative basophil count from baseline to finish line. This decrease was not due to caffeine supplementation and did not differ between males and females. The baseline relative

basophil count was above the recommended range ($> 1\%$), but, finish line values returned to normal, and were within the recommended range of 0-1% of the total WBC count. The relative basophil count did not influence the overall time to complete the triathlon.

Given the known large day-to-day variation in these parameters, the small differences reported here are likely not of clinical relevance.

5.2.3 Plasma lactate

Lactate is a by- or waste product of oxygen-independent glycolysis in the muscle. When glucose is broken down to form pyruvate to provide energy in an oxygen-independent environment, lactic acid is formed. This readily dissociates to form lactate and hydrogen ions. Lactate can then be used during oxygen dependent energy metabolism (as in the case with endurance exercise or triathlons) to produce glucose again in the liver *via* the Cori cycle (5). Therefore, lactate, which is derived from muscle, can be a very important gluconeogenic antecedent and a valuable metabolic intermediate, rather than just being a by-product of oxygen-independent glycolysis (222). In a review by Robergs *et al.* (2004) on the biochemistry of exercise-induced metabolic acidosis, it was concluded that, “*an increased lactate production coincides with cellular acidosis and remains a good indirect marker for cell metabolic conditions that induce metabolic acidosis. If muscle did not produce lactate, acidosis and muscle fatigue would occur more quickly and exercise performance would be severely impaired*” (223).

All lactate levels, with the exception of baseline measurements in all subjects and the male caffeine group were, as expected during exercise, were well above the reference range for blood lactate levels (> 1.0 - 1.8 mmol/l). There were significant differences between the caffeine and placebo group in all subjects during transition (cycle \rightarrow run) and 9 and 12 minutes after the finish line, as well as in the male group during transition (cycle \rightarrow run) and at 3 minutes after the finish line. There was an overall significant difference between lactate levels measured at various time points in the caffeine and placebo groups. The caffeine group had lower baseline values, which increased more during transition and 3 minutes after the finish line, and then showed a steady decline but were still higher than the values in the placebo group. In the placebo group there were higher baseline values, which increased more gradually during exercise, then declined faster after the finish line and subsequently increased again from 12 and 15 minutes after exercise. There were no differences in lactate measurements between males and females and none of the measurements taken influenced the overall time to complete the triathlon.

These results are in agreement with research conducted in 1985 by Gaesser *et al.* on incremental exercise. These authors found that the accumulation of blood lactate is not delayed by caffeine supplementation, and it may even increase the onset of blood lactate accumulation (224). In contrast, other studies examining the effect of energy drinks (containing caffeine) on blood lactate levels found an increase in blood lactate levels two minutes before and two minutes after Bruce treadmill tests, but this increase was not due to the energy drink (225). McNaughton 2008 reported an increase in blood lactate levels at the end of a one hour time trial performance in six male cyclists, irrespective of whether caffeine supplementation was administered or not (88).

Therefore, caffeine increases the production of lactate, as seen in the results of the current study, where in the caffeine group there was lower baseline levels, which increased more during transition (cycle → run) and three minutes after the finish line, where after it showed a steady decline after finish line compared to the placebo group that had higher baseline levels, levels increased more gradually during exercise, then declined faster at the finish line and then displayed an upward curve from 12-15 minutes after exercise. The more drastic increase in lactate levels during transition and directly after exercise, can be used as a fuel source by converting lactate to glucose (gluconeogenesis) in the liver or to alanine and then to glucose in the muscle, thereby maintaining blood glucose levels, reducing fatigue and improving exercise performance. The steadier decline seen after exercise can also help to maintain blood glucose levels immediately after exercise.

It appears in the present study that the main mechanisms of action of caffeine as an ergogenic aid is the effect on the central and autonomic nervous systems, as indicated by an increased cortisol response following exercise in the caffeine group, as well as increased leukocytosis, neutrophilia and lymphocytosis. The increased plasma lactate levels also suggest that the main mechanism of action is due to direct stimulation of the CNS or due to the adrenergic effect of caffeine supplementation. However, the latter was not measured in the present study.

If we therefore revisit Figure 2.2 (Figure 5.1), we can conclude that the present study had the most profound effect on increased cortisol, leukocytes and plasma lactate (blocks shaded in blue) and that the effect of increased cortisol or the physical stress of exercise negated the effect of caffeine supplementation on testosterone and prolactin levels (blocks shaded in pink).

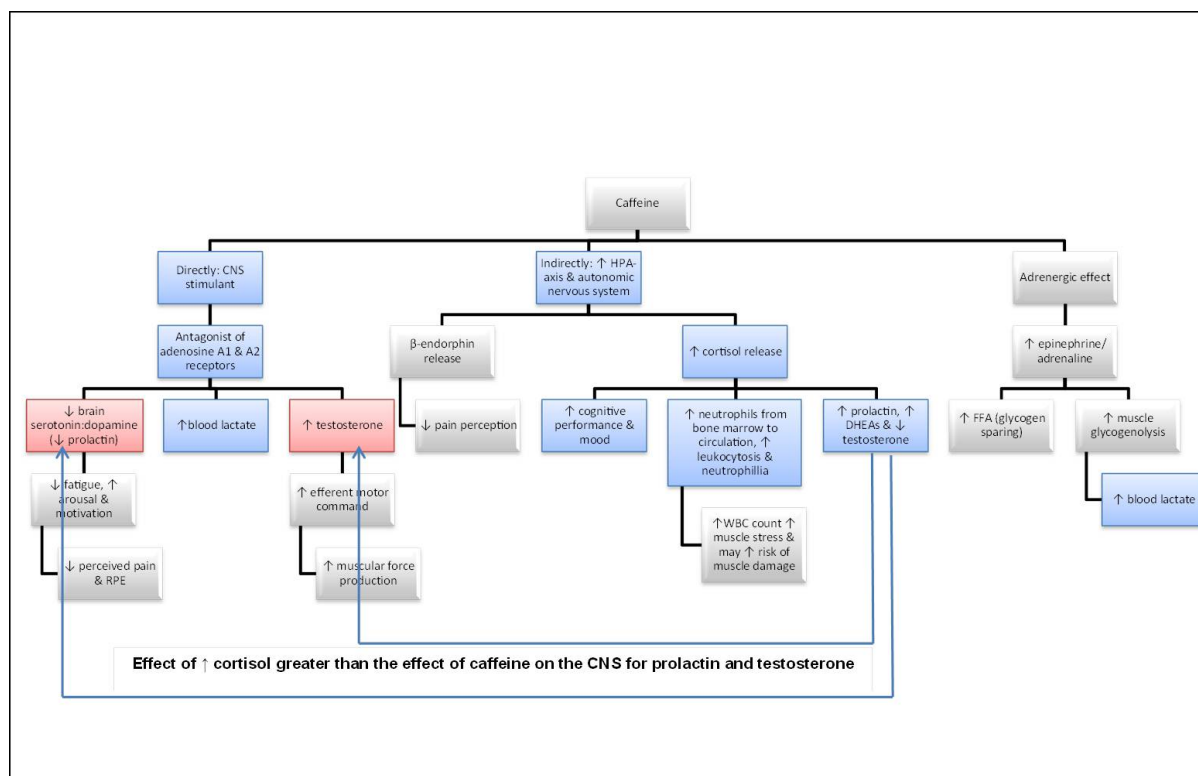


Figure 5.1 Mechanisms of action of caffeine supported by the present study's results

5.3 Factors influencing the ergogenic effect of caffeine supplementation

5.3.1 Lifestyle

5.3.1.1 Caffeine habituation

Habitual caffeine intake

In the present study, the mean habitual intake of the triathletes was ± 400 mg/day. This group of subjects can thus be classified as high habitual caffeine consumers (≤ 50 mg/day is considered low intake, whilst ≥ 300 mg/day is considered high habitual intake) (72) when interpreting the mean habitual caffeine intake. It was however noted that the SD was higher than the mean habitual intake, indicating that the subjects had a wide range of habitual caffeine intake. However, the mean intake is high in terms of the South African population, as it has been reported that the average caffeine consumption in South Africa is only 40 mg/person/day (57).

The subjects were asked to abstain from caffeine-containing products for 14 days before each trial. Upon analysis of baseline caffeine levels, it is evident that the athletes adhered to this pre-race stipulation.

The current habitual caffeine intake of this group of athletes does not compare to surveys done on other athletes. The present study population had a much higher habitual caffeine intake than British athletes (70). Chester *et al.* (2008) investigated caffeine consumption amongst British athletes following changes to the 2004 WADA List of Prohibited Substances and found that more cyclists used caffeine, compared to track and field athletes. The majority of the cyclists, nevertheless, consumed less than 200 mg caffeine per day (70).

The main sources of caffeine in the study by Chester *et al.* (2008) were coffee, pharmaceutical preparations and energy gels/bars/powders. The most common reasons given for using caffeine included subjects enjoying the taste, wishing to potentially improve sporting performance and using caffeine for work/study purposes (for example to stay awake or improve concentration) (70). A study by Desbrow *et al.* (2006) on caffeine use and awareness by triathletes competing at the 2005 Ironman Triathlon World Championships revealed that the majority of triathletes planned to use caffeine-containing substances prior to or during the race. The most popular of these substances were cola drinks, caffeinated gels, coffee and NoDoz[®] tablets (226, 227).

Although the mean daily caffeine intake found in the present study is higher than that reported by Chester *et al.* (2008), it is comparable to the self-reported mean daily intakes of caffeine in six Canadian recreational male athletes (running or cycling five times a week for more than three months). This was found to be as high as ± 700 mg/day (26).

3.3.1.2 Influence of the pre-event meal on caffeine absorption

The pre-event meal in the present study was consistent between the caffeine and placebo groups. This was expected as subjects were asked to repeat the same dietary intake before and on the day of both triathlons. The pre-event meal had no influence on the absorption of caffeine and therefore plasma caffeine levels were not affected by the pre-event meal.

5.3.2 Menstrual patterns, oral contraceptive use and menopause

Only eight percent ($N = 1$) of the females in the current study population experienced oligomenorrhea in the preceding 12 months. Five of the 12 females (42%) were post-menopausal. Although the females had a low (< 45 kcal/kg FFM)_{est}EA, it was still > 30

kcal/kg FFM and they seemed to reach their energy needs when looking at their energy intake. Menstrual dysfunction has been shown to occur at estEA levels of < 30 kcal/kg FFM (4). Subtle changes in menstrual function that can only be evaluated with urine or blood samples, such as luteal phase defect (LPD) and anovulation can also occur during transient times of energy deficit. LPD cycles in athletes are associated with a metabolic hormone profile indicative of a hypometabolic state. This is similar to that observed in amenorrheic female athletes (228).

All of the pre-menopausal females in the current study used oral contraceptive medication. The prevalence of oligomenorrhea and amenorrhea might have been higher had they not used oral contraceptives to regulate their cycle.

When studying the effect of caffeine supplementation on exercise or sporting performance, it is important to note the phase of menstrual cycle (follicular, luteal or post-menopausal), as well as the possible use of oral contraceptive medication. Lynch *et al.* (1998) studied the effect of oral contraceptive use and the phase of the menstrual cycle on exercise performance (running) in 15 women. The authors found that exercise performance does not vary between the mid follicular phase and the late luteal phase of the menstrual cycle and it is not affected by oral contraceptive use (229). However, the effect is seen when caffeine supplementation enters the equation. Although some studies (230) have reported that the menstrual cycle or gender does not affect the pharmacokinetics of caffeine, others have reported the effects of the abovementioned parameters on the pharmacokinetics of caffeine. Lane *et al.* (1992) reported that the elimination of caffeine can be decreased in the luteal phase of the menstrual cycle, although the extent of this may be small (62). Delayed elimination, prolongs the half-life of caffeine, which could lead to increased plasma concentrations of caffeine and a greater risk of side-effects.

In the present study, one of the research objectives was to determine if the phase of menstrual cycle (luteal or follicular) influenced plasma caffeine levels. The researcher determined the phase of the menstrual cycle by calculating the days since the last menstruation and using the self-reported length of the menstrual cycle. This is a limitation, because the phase of the menstrual cycle was self-reported and not measured. There were also no statistically significant differences in the overall time to complete the triathlon, regardless of whether the female athletes were in the luteal or follicular phase. This might have been a true effect or a type two error since the phases of the menstrual cycle might have been misreported. It was, however, interesting to note that although this result did not reach statistical significance, the females receiving caffeine supplementation during the

follicular phase had an overall faster time to complete the triathlon compared to the females receiving the placebo (4% decrease in time). Although it cannot be unequivocally proven in this study, the abovementioned finding suggests that during the follicular phase, caffeine supplementation has a more beneficial effect on sporting performance than during the luteal phase. This could possibly be due to fewer side-effects of caffeine supplementation (that could potentially impact exercise performance) being experienced or enhanced caffeine metabolism during the follicular phase.

After observing a non-statistically significant 4% decrease in triathlon times for the females in the follicular phase compared to the luteal phase of the menstrual cycle, the researcher analysed the data according to individuals (Figure 5.2)

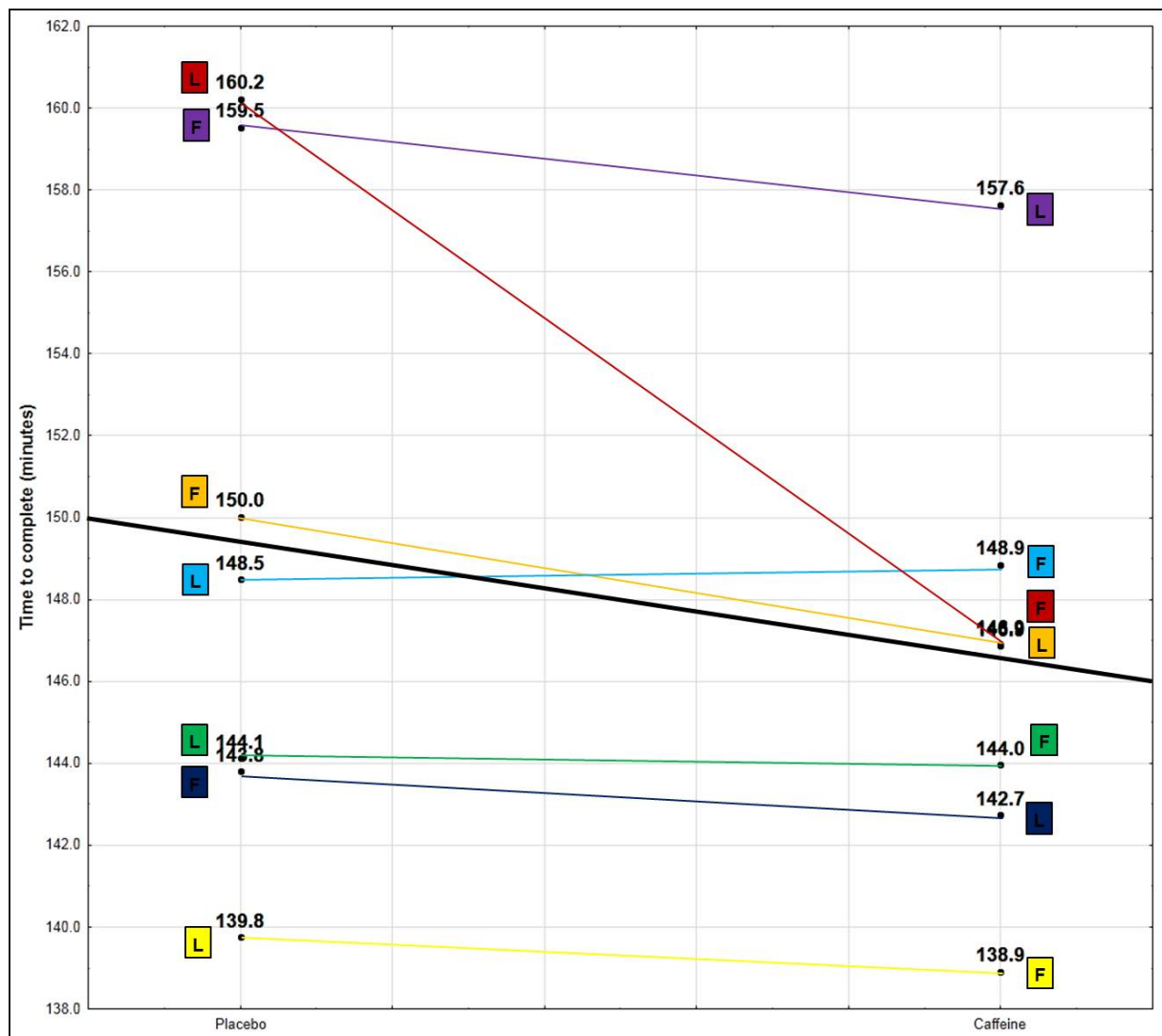


Figure 5.2 Influence of the phase of menstrual cycle on the time to complete the triathlon in the caffeine and placebo groups

F: Follicular phase, L: Luteal phase, black line indicates mean time to complete in caffeine and placebo groups

It is evident from the individual graph presented in Figure 5.1 that all, except for one female had a decreased time to complete the triathlon when receiving caffeine supplementation during the follicular phase of the menstrual cycle. If one therefore has to draw a conclusion or make a recommendation one can say caffeine may be more effective in the follicular phase, however, this remains to be substantiated.

Caffeine metabolism may be altered / inhibited in post-menopausal women receiving hormone replacement therapy (192). In the current study, menopause did not influence plasma caffeine levels. It was, nevertheless, interesting to note that although there was no statistically significant difference, the post-menopausal group completed the triathlons on average 14-17% (20-30 minutes) slower than the females who had not yet reached menopause, irrespective of whether or not caffeine supplementation was given. This could have possibly also been related to the age of the post-menopausal females, who were typically older than the pre-menopausal females. However, this observation is less likely as one of the females who were post-menopausal and > 50 years, won the female race and came third overall.

All seven pre-menopausal females used oral contraceptive medication, mainly for birth control. Subjects also indicated that they were using an oral contraceptive to regulate their cycle in addition to using it for birth control. As with hormone replacement therapy, oral contraceptive use has been known to interfere with caffeine metabolism and chronic use leads to increased plasma caffeine concentrations (63, 193). The use of oral contraceptive medication in the present study did not influence the plasma caffeine levels measured during transition (cycle → run) or at the finish line in the caffeine or placebo groups, nor did it have an effect on the overall time to complete the triathlon, irrespective of whether or not caffeine supplementation was given.

Summarising the influence of menstrual cycle, menopause and oral contraceptive use on the ergogenic effect of caffeine supplementation, the self-reported phase of the menstrual cycle or oral contraceptive use had no effect on caffeine metabolism or the overall time to complete the triathlons. Although not statistically significant, but clinically relevant, we observed faster times when the women were in the follicular phase of the menstrual cycle whilst receiving caffeine supplementation, when compared with the luteal phase of the menstrual cycle. Post-menopausal women also completed the triathlons on average slower than pre-menopausal women, irrespective of caffeine supplementation. Oral contraceptive use had no effect on caffeine supplementation or time to complete the triathlons.

5.3.3 Genetic analysis

5.3.3.1 *CYP1A2*1F (rs762551) single nucleotide polymorphism (SNP)*

The *CYP1A2*1F* polymorphism has been associated with altered enzyme activity (160). Carriers of the *CYP1A2*1F* C-allele are “slow” caffeine metabolizers, whereas individuals who are homozygous for the A-allele (ancestral type) are “rapid” caffeine metabolizers (158, 231-233).

In the current study, the frequency of the C-allele (0.29, Table 4.12) associated with “slow” metabolism of caffeine was similar to the frequency reported for a Turkish population (0.27) (234), although slightly lower, than *CYP1A2*1F* frequencies, reported in Caucasian populations, such as in 114 British individuals (0.34) (160, 201), 495 German individuals (0.32) (235) and 236 Germans (0.32) (233), and much lower than the frequency reported in 193 Swedes (0.57) (160, 236), Japanese individuals (0.63) (237) and African populations, such as Tanzanians (0.51) and Zimbabweans (0.43) (160, 238). Genotype frequencies of C-allele carriers in this study (38%) were also lower compared to frequencies reported in 553 young Caucasian individuals (59%) (232), 236 German individuals (54%) (233) and 35 male recreational competitive cyclists (54%) (165).

In the current study, the *CYP1A2*1F* C-allele did not have a significant effect on plasma caffeine levels throughout the triathlons in the caffeine or the placebo groups, nor did it have any effect on the overall time to complete the triathlon. Even though this result was not statistically significant, those subjects with the A→C substitution had decreased plasma caffeine levels during transition (cycle → run) and at the finish line. This is in contrast with studies describing that individuals with an A→C substitution are “slower” metabolizers of caffeine (and therefore have increased plasma caffeine levels) compared to individuals who are homozygous for the *CYP1A2*1F* A-allele who are “rapid” metabolizers of caffeine (158).

A recent study by Womack *et al.* (2012), evaluated *CYP1A2*1F* (rs762551) and its effect on 6 mg/kg body weight caffeine supplementation and sporting performance in 35 well-trained male cyclists. These authors concluded that the time to complete a 40 km cycle was shortened in the A/A homozygotes, illustrating “rapid” caffeine metabolism and therefore increased exercise performance (165, 239). This is in keeping with current literature on this SNP, indicating that individuals who are homozygous for the *CYP1A2*1F* A-allele are “rapid” metabolizers of caffeine (158, 165, 240). Womack *et al.* (2012) speculated that the ergogenic effect found in “rapid” metabolizers of caffeine may be due to the fact that two of the metabolites of caffeine, paraxanthine and theophylline have higher binding affinities with

adenosine receptors. Therefore, if an individual metabolized caffeine more rapidly, paraxanthine and theophylline is produced more rapidly, leading to the enhanced ergogenic effect (165).

5.3.3.2 CYP1A2*1D (rs35694136) single nucleotide polymorphism (SNP)

*CYP1A2*1D* is one of the most extensively studied polymorphisms and has been associated with lower CYP1A2 enzyme activity. The frequency of the -2467T deletion is lower in Caucasians compared to Asians and Africans (160). The allelic frequency of *1D deletion allele is 0.05-0.24 in Caucasians (160, 201). The -2467delT appears much more frequently in Japanese populations (0.42-0.44) (160, 237, 241), in Egyptians (0.40) (160) and is very high in Turkish populations (0.92) (160, 242). In the present study group, which consisted solely of Caucasians, the -2467delT frequency (0.08) corresponded with that reported for other Caucasian populations. There were no significant differences in the plasma caffeine levels measured during transition (cycle → run) or at the finish line, regardless of the presence of -2467delT. The presence of this deletion also did not have an influence on the overall time to complete the triathlon.

5.3.3.3 CYP1A1-CYP1A2 (rs2472297-T and rs2470893-A) and the variant near the AHR gene (rs698865-T)

Allele frequencies for all three SNPs, rs247229-T, rs2470893-A and rs698865-T, corresponded to that determined in Caucasian populations (<http://www.ncbi.nlm.nih.gov/projects/SNP/>).

CYP1A1 and CYP1A2 activity is co-regulated. Although the effects of CYP1A2 on caffeine metabolism have been studied extensively and is well established, the role of CYP1A1 in caffeine metabolism has not been verified (163). The aryl hydrocarbon receptor (AHR) induces members of the CYP1A1 and CYP1A2 family and is known to induce the activity of *CYP1A1* and *CYP1A2* by binding to DNA in the region between these two genes (163).

CYP1A2 enzyme activity is increased in habitually high coffee consumers. It is known that lower activity of this enzyme can increase caffeine toxicity as this is the key enzyme responsible for caffeine metabolism. However, in the case of rs247229 and the SNP near the *AHR* gene, individuals can consume more caffeine without experiencing toxic or negative side effects, due to increased CYP1A1 and CYP1A2 enzyme activity; this increased enzyme

activity enhances the rate of caffeine metabolism, which leads to lower plasma concentrations of caffeine (163).

In the current study, rs2472297-T, rs2470893-A alleles and the variant near the *AHR* gene (rs6968865-T) did not have any effect on plasma caffeine levels during transition (cycle → run) or at the finish line. None of these variants influenced the overall time to complete the triathlon.

As discussed in the literature overview, a large interindividual variation in expression and activity of CYP1A2 exist. This has a direct influence on the clearance of drugs, such as caffeine, which is extensively metabolized by CYP1A2. Various genetic factors, such as epistasis, i.e. the effects of one gene being modified by several other genes, epigenetic factors, i.e. heritable changes in gene expression or cellular phenotype caused by mechanisms other than changes in the underlying DNA sequence, subcellular factors and intranuclear positioning of regulatory genes as well as environmental factors (such as smoking and use of medication) influence the expression and activity of CYP1A2 (162). This can partially explain this interindividual variability and the inconsistencies reported in genetic associations. It is unlikely that the metabolic phenotype can be predicted from a single SNP or haplotype. There are also distinct interethnic variations in CYP1A2 activity (162).

Therefore, in this study, neither *CYP1A2* gene polymorphism nor a variant near the *AHR* gene influenced caffeine supplementation's effect on triathlon performance. This could have been due to the limited sample size or the fact that the effect is not easily discernible during a triathlon. In the present study, the "slower" metabolizers of caffeine did not experience a lesser effect of the caffeine supplementation.

In conclusion, habitual caffeine intake did not affect caffeine ergogenicity in the present study as subjects abstained from caffeine intake for two weeks prior to both trials. It appears that caffeine supplementation may be more effective during the follicular phase of the menstrual cycle and that genetics does not influence caffeine supplementation's ergogenicity, although these results remain to be substantiated in a larger cohort.

5.4 Factors influencing triathlon performance

5.4.1 Medical history and supplement use

The overall health of the study population was good, with few reported chronic diseases or chronic medication usage. Supplement use amongst the athletes was rife, with 85% of the

subject group taking some form of dietary supplementation. The most common reasons given for the use of supplements, namely to prevent illness, because of inadequate diet and to enhance exercise performance, coincided with the type of supplements used most frequently (multivitamins- and mineral formulations, and energy drinks and bars). The prevalence of supplement use is higher in the present study, compared to a survey by Dolan *et al.* (2011) on 401 triathletes. The authors found that 54% of triathlete's surveyed use supplements, of which 21% use caffeine supplementation (243).

The supplements most frequently consumed by the current study population are relatively low-risk supplements (i.e. low risk for potential adverse physiological effects, testing positive for banned substances or the potential for dependence, addiction or abuse). It is important to note that supplements and sports foods are widely used by physically active individuals, most often to improve exercise performance and overall health, or to improve or change parameters of body composition. It is important when evaluating and prescribing the use of supplements to judiciously consider the risk/benefit ratio of using a particular supplement.

In South Africa, the dietary supplement market is currently poorly regulated by the Medicines Control Council. The widespread use of supplements, the fact that many products are readily available for sale directly to customers over the Internet and the possible contamination of dietary supplements, both directly and indirectly, further increase the potential for irrational drug use. The safety, purity, efficacy and proven benefit of supplements should be evaluated before use. Problems encountered with supplements and complementary medicines include, but is not limited to the following: i) all active pharmaceutical ingredients in the formulation may not have been identified and the dosages of these measured accurately and standardised; ii) these dosages may not be appropriate (for example high dosages of certain vitamins); iii) it can be difficult for consumers to assess whether doses are appropriate because of inadequate labelling; iv) lack of awareness amongst consumers about the need to check dosages and ingredients; v) the misconception that if something can be obtained without a prescription it is "safe"; vi) consumers may be taking more than one product and there may be duplication of ingredients which could result in toxicity; vii) a supplement could be inappropriate for a particular person due to co-morbidities or other medical conditions; and viii) there may be drug interactions between supplements and other medication taken.

Currently, the World Anti-Doping Association (WADA) does not distinguish between deliberate cheating and inadvertent doping and the responsibility is solely the athlete's (170, 186).

Finally, although there are some supplements that may have a beneficial effect on athletic performance, for example carbohydrate supplements, it should be noted that no amount of supplementation can compensate for a poor diet (170).

To summarize, the subjects had an overall good medical history, except for the prevalence of low BMD and osteopenia, which was found during the data collection. Supplement use was rife amongst the subjects. Supplement use included low risk supplementation such as carbohydrate-electrolyte solution, liquid meal replacements and multivitamin –and mineral supplements. The supplements used were quantified with the dietary intake, and even with supplement use, the dietary intake of this group was still inadequate for total energy and CHO intake as discussed below.

5.4.2 Full blood count

The heamatocrit and haemoglobin levels were also measured to detect any changes in plasma volume. The red blood cell counts, haemoglobin level and related indices reveals whether an individual is anaemic, whilst the white blood cell (WBC) count indicates whether an infection is present (5).

Red blood cells, also known as erythrocytes, are mainly responsible for transporting oxygen and carbon dioxide between lungs and tissues. Haemoglobin is the oxygen-carrying pigment of RBC and the heamatocrit is the total blood volume packed with red blood cells. The heamatocrit is also known as the ratio of red blood cells to plasma and is expressed as a percentage of total blood volume. Platelets meanwhile are instrumental in coagulation or blood clotting (5).

Interpretation of the red blood cell (RBC) count in isolation is not recommended or clinically significant. This needs to be interpreted in combination with the haemoglobin and heamatocrit values, and other components of the RBC count (217). RBC values obtained in the present study fell within the reference ranges ($4.5\text{--}6.6 \times 10^{12}/\text{l}$ for males and $3.8\text{--}5.8 \times 10^{12}/\text{l}$ for females). There was a significant reduction in RBC count from the baseline to the finish line, but this difference was not due to caffeine supplementation. There were no differences between RBC count values in males and females, and the RBC count did not influence the overall time to complete the triathlon.

A decrease in haemoglobin is clinically used to define or diagnose anaemia, although further analyses of components of RBC are necessary to confirm this. Increased haemoglobin

levels are indicative of changes in plasma volume due to dehydration, alcohol use, smoking and the use of diuretics (217). In the present study, the haemoglobin values measured at baseline and at the finish line fell within the reference range (13.0-18.0 mg % for males and 11.5-16.5 mg % for females). Therefore, the athletes in the current study were unlikely to have anaemia, especially as their iron intake as discussed with the dietary intake, was also above the DRI.

There were no differences in values obtained at baseline and at the finish line, irrespective of caffeine supplementation and gender. The haemoglobin levels also had no influence on the overall time to complete the triathlon.

In the present study, all heamatocrit values fell within the reference range (40-54% for males and 38-47% for females). There was a significant decrease in the heamatocrit values between the baseline and the finish line in all subjects, and the male and female caffeine groups. In the placebo group, there was only a significant decrease between baseline and finish line values in the male group. The decreases observed between the baseline and finish line values in the caffeine group were not due to caffeine supplementation and were present irrespective of gender. The heamatocrit values did not influence the overall time to complete the triathlon.

A low platelet count, or thrombocytopenia, increases the risk of bleeding. This low count can be present due to viral infections, idiopathic causes, liver disease, medication, pregnancy and autoimmune diseases. Increased platelet counts or thrombocytosis appear in reactive conditions such as infection, inflammation, pregnancy and iron deficiency (217). The reference range for the platelet count is $150-400 \times 10^9/L$.

The platelet count of all subjects, and the male and female groups fell within this range at baseline and at the finish line. There was, however, a significant increase in the platelet count from baseline to the finish line in all these groups. This increase was not due to caffeine supplementation and may instead have been due to exercise-induced infection or inflammation. This is also supported by increases in WBC count and more specifically the neutrophil and lymphocyte counts, although the latter was more significantly higher in the caffeine compared to placebo group. The abovementioned increase in the platelet count did not differ between genders. The platelet count did not influence the overall time to complete the triathlon.

5.4.3 Mood state

Subjects completed a shortened version of the POMS questionnaire during the week before T1 and T2. This was completed to determine whether or not subjects had adequately recovered between the two exercise trials. When comparing the total scores, there were no differences between T1 and T2, which suggests that the subjects had adequate time between the two trials to recover fully. There were also no differences between the vigour and tension scores between T1 and T2. There was, however, a difference between the fatigue scores the week before T1 and T2, with females being more fatigued the week before T1 and males being more fatigued the week before T2. However, none of these scores measured during the week before T1 or T2 influenced the overall time to complete T1 or T2. This confirms that subjects had recovered adequately between the two trials.

To summarise, the mood state of the athletes was measured to determine if the athletes had sufficiently recovered between T1 and T2 and to measure the effect of caffeine supplementation on mood state. The 14 days between T1 and T2 allowed sufficient time for the athletes to recover fully between T1 and T2.

5.4.4 Energy- and nutrient intake two days before as well as dietary strategies followed on race day

5.4.4.1 Dietary intake two days before T1 and T2

Energy and estimated energy availability (_{est}EA)

The daily energy requirements for athletes doing moderate intensity training 2-3 hours per day, 5-6 times per week, such as the training undertaken in this study population, is 50-80 kcal/kg BW (170). The American Dietetic Association (ADA) and the American College of Sports Medicine (ACSM) recommends including enough energy to maintain optimal body weight and support exercise performance (183). The total energy intake of the athletes in this study group is therefore slightly low according to the abovementioned guidelines. The average energy intake of T1 and T2 was 37.7 kcal/kg BW for all subjects and 34.9 kcal/kg BW and 40.9 kcal/kg BW for males and females respectively. Therefore, the males had a much lower energy intake compared to the females.

The total energy intake (kcal/kg BW) was lower in males than in females. There was no significant difference between the total energy intake two days before T1 and two days before T2, suggesting adherence to the experimental protocol, as subjects were asked to

consume the same foods that they would usually eat before a race and to repeat this before T1 and T2. The total energy intake two days before T1 and T2 also had no influence on the overall time to complete T1 or T2.

The energy intake, when measured in kcal/kg BW as mentioned above was insufficient according to the guidelines. This is important to note, as athletes need more energy- and macronutrients in proportion to their body weight expressed in kilograms than sedentary individuals. Therefore, according to the ADA, “expressing energy- and macronutrient needs in terms of grams per kilogram body weight is a practical method to document these needs” (183).

However, when comparing the energy intake in kilojoules (kJ) per day with the energy intake found in other studies, it seems similar. The athletes in the present study had an average energy intake of 10737.3 kJ, with males 10945.2 kJ and females 10500.0 kJ/day. The total energy intake is the same as reported in the literature on triathletes for females (Worme *et al.* (1990) 9058 kJ and Potgieter *et al.* (2011) 9004 kJ, but lower for males (Worme *et al.* (1990) 11591 kJ and Potgieter *et al.* (2011) 14535 kJ (71, 244).

As discussed in the literature review, the International Olympic Committee (IOC) recommended in its 2003 and 2010 consensus statement on sport nutrition that estimated energy availability ($_{est}EA$) is a better indication of an athlete's energy status than energy balance (4). Calculating $_{est}EA$ can identify those athletes at risk of being energy deficient. Several physiological responses are adversely affected by a low $_{est}EA$ (< 30 kcal/kg FFM). Physiological responses known to be affected by a low $_{est}EA$ include a physically active female's menstrual cycle, which could result in reproductive dysfunction (4); the immune system functioning (4, 245, 246) and bone health (247). The $_{est}EA$ should therefore be at least 30-45 kcal/kg FFM in order to sustain exercise and promote good health (4).

The group of athletes in the current study population had a mean $_{est}EA$ of less than 30 kcal/kg FFM, with males between 20-25 kcal/kg FFM and females between 33-37 kcal/kg FFM. The range of $_{est}EA$ was wide, with only 30.7% (8/26) athletes having an $_{est}EA$ > 30 kcal/kg FFM. Sixty-nine percent (18/26) of athletes had a low $_{est}EA$, of which 44.4% (8/18) had an $_{est}EA$ < 20 kcal/kg FFM and 16.6% (3/18) an $_{est}EA$ < 10 kcal/kg FFM. Furthermore, 78.6% (11/14) of the males were identified with a low $_{est}EA$ compared to 58.3% (7/12) of the females.

This was interesting to note, as the athletes were supposed to have a high energy intake (possibly due to CHO-loading) and low exercise energy expenditure as they were tapering down their exercise before the races.

However, the low $_{est}EA$ could have been due to various other factors. First, although the $_{est}EA$ in the present study was low when calculated for “low” or tapering training days and the subjects were instructed to refrain from exhaustive exercise for the two days leading up to the races, the ideal time period to calculate the $_{est}EA$ is seven days to account for high- and low volume training days. Second, the activity of daily living was calculated in the formula to determine $_{est}EA$. The METs value used to represent general daily living is based on the assumption that these athletes typically would do, as they did not keep an exercise log of daily activities, but only of planned exercise. Third, the record keeping of their planned exercise and food intake was done with a food record. Although the food record have numerous advantages, such as not relying on an individual’s memory and that it can provide a detailed account of a subject’s intake and exercise habits, it does have a high subject burden and subjects may have changed their usual eating and training routines because they knew they were being monitored.

With this said, the $_{est}EA$ in the present study was low for the total group. Differences found between $_{est}EA$ of males and females can be attributed to the females reporting a higher energy intake compared to males. A low $_{est}EA$ is reversed by increasing energy intake to match daily energy expenditure and reduced training is not always needed (206). The reason for this is because the consequences of a low $_{est}EA$ are not necessarily due to stress or exercise, but rather the energy cost of exercise. Therefore, increasing energy intake or decreasing training, can alone, or in combination lead to more favourable $_{est}EA$ and ultimately decrease the negative consequences associated with a low $_{est}EA$ (206).

Carbohydrate

CHO requirements for physically active individuals (moderate-high intensity training, 2-3 hours per day, 5-6 days per week endurance programme and moderate-high intensity, 1-3 hours/day) range between 5-8 g/kg BW (170) and 6-10 g/kg BW (169, 183). However, as dietary intake in the present study was measured in the two days before T1 and T2, it was expected that the athletes would be CHO loading, as the events lasted longer than 90 minutes. CHO loading is a strategy designed to optimize muscle glycogen stores prior to endurance events lasting longer than 90 minutes (168). This strategy has been proven to enhance performance during endurance exercise, work output and immunity; and to maintain muscle tissue stores (174). The CHO loading regime is complemented by including

a pre-event meal rich in CHO before the race, as well as optimal CHO intake during the event (174). CHO loading requirements range from 8-10 g/kg BW for 1-3 days prior to the event (174) to 7-12 g/kg BW for 24 hours prior to the event (169).

The athletes in the present study did not achieve the abovementioned recommended daily CHO requirements and also did not meet the recommendations for CHO loading, as they had a mean intake of 4-5 g/kg BW for all subjects, < 4 g/kg BW for the males and 4.5-5.5 g/kg BW for the females. This is of concern, as literature has indicated the significant value of increasing CHO intake in the days leading up to an endurance event and its effect on increased endurance performance during events lasting > 90 minutes by 2-3% over a set distance, due to the super-compensation of muscle glycogen stores (168, 169, 248).

Although not measured in the study, a subjective observation in the present study population was that the male athletes were more aware of their body composition and in general more focussed on dietary intake. This could in part explain why the males had a lower dietary intake compared to the female group. The lower CHO intake in the male group is consistent with results from Frentzos *et al.* (1997) amongst elite triathletes (4.0 g/kg BW) (240), but differed from results reported by Potgieter *et al.* (2011), 5.3 g/kg BW (61), Nogueira *et al.* (2004), 4.5-11.3 g/kg BW (241) and Worme *et al.* (1990), 5.1 g/kg BW (235). The CHO intake of the females were in keeping with CHO intakes found by Nogueira *et al.* (2004) in endurance athletes, 4.4-7.2 g/kg BW (241) and Worme *et al.* (1990) in 21 female triathletes, 4.9 g/kg BW (235), but higher than the CHO intake of triathletes reported by Potgieter *et al.* (2011), 3.5 g/kg BW (61).

If CHO-loading requirements are not met, athletes should nevertheless aim to reach the requirements for daily CHO intake. The CHO intake of the whole group, and of the males and females, did not differ between T1 and T2, but it was evident that the CHO intake positively influenced the overall time to complete the triathlon (increased intake improved time to complete), reaching statistical significance in the male group for T2. It is interesting to note that the CHO intake influenced the performance of the male subjects, who generally had a lower CHO intake. Therefore, a practical recommendation to these athletes would be to increase their CHO intake in the two days leading up to a race, as this can improve their triathlon performance.

The DRI of fibre is 20-30 g/day (176). The group had a fibre intake of 22-27 g/day, which falls within the recommended reference range, with males having slightly higher fibre intake than females. This is in keeping with the previous results from Potgieter *et al.* (2011), where

the male group had a fibre intake of 26.7 g/day and the female group 22.2 g/day (71). Fibre intake did not differ before T1 and T2, but influenced the overall time to complete both T1 and T2. A higher fibre intake was associated with a shorter time to complete the triathlons, especially T2. It does however seem that the effect was rather due to the intake of CHO, than the intake of fibre, as those that chose to eat CHO, chose food items high in fibre such as oats and brown bread. There is also evidence from the literature that a high CHO diet can reduce *ad libitum* energy intake, due to its appetite suppressing nature. This appetite reducing effect might be related to its fibre content (4). The relative high fibre intake of our athletes might have had an appetite suppressive effect contributing towards low energy and CHO intakes. This is, however, purely speculative as we did not measure appetite or hunger ratings. The high fibre intake is also in contrast with recommendations that CHO-loading should contain mostly high glycaemic index CHO, low fibre/residue CHO-rich foods (168, 169, 174). This association between a higher fibre intake and a decreased time to complete the triathlons could also have been a coincidence because of the many variables studied and the relatively small sample size.

Protein

The protein intake in the present study ranged from 1.5 – 2.0 g/kg BW, and was higher in the female group (1.7 – 2.0 g/kg BW) compared to the male group (1.4 g/kg BW for both T1 and T2). It is recommended that athletes performing moderately intense training have a protein intake ranging from 1.0-1.5 g/kg BW (170, 249) or 1.2 – 1.7 g/kg BW (183). The protein intake in the total group was therefore sufficient, if not slightly high, especially for the female group. The protein intake did not differ between T1 and T2. When comparing the protein intake of the current study group to the habitual protein intake of triathletes reported in the literature, it falls within the same ranges as reported by Potgieter *et al.* (2011), (1.95 g/kg BW for males and 1.2 g/kg BW for females) (61), Nogueira *et al.* (2004), (1.2-2.0 g/kg BW for males and females) (241) and Worme *et al.* (1990), (1.4 g/kg BW for males and females) (235).

Fat

Dietary fat requirements are similar in physically active individuals compared to their non-physically active counterparts. It is important for athletes to consume adequate amounts of fat in order to achieve optimal health, replenish intramuscular triacylglycerol stores after exercise and to ensure adequate intake of essential fatty acids and the fat soluble vitamins A, D, E and K (170, 183). According to the ISSN, fat requirements range from 30-50% of total energy and can also be calculated in g/kg BW (0.5-1.5 g/kg BW). It is recommended that when an athlete is attempting to reduce his/her body weight, fat intake should be

decreased to 0.5-1.0 g/kg BW (170). The ADA and ACSM recommend a daily fat intake of 20-35% of total energy intake, and that this fat intake should consist of 10% monounsaturated fatty acids, 10% polyunsaturated fatty acids and 10% saturated fatty acids (183).

The group of athletes in the present study had a daily fat intake of 1.2-1.4 g/kg BW (30-35% of total energy), with males at the lower end and females towards the higher end of this range. This is in accordance with the abovementioned daily fat intake recommendations of the ISSN, ADA and ACSM. The fat intake did not differ between T1 and T2, nor did it have an influence on the overall time to complete T1 or T2. The fat intake of the current study group compares to the results from Potgieter *et al.* (2011), which found a total fat intake of 29.8% for females and 34.6% for males (71).

Alcohol

The alcohol intake differed significantly before T1 and T2 in the male group. However, the alcohol intake in the male group before T1 and T2 still fell below the prudent dietary guidelines of 10-20 g/day. Alcohol intake also did not influence the overall time to complete T1 or T2. The average alcohol intake of T1 and T2 in the present study (8.5 g/day for all subjects, and 12.3 g/day and 1.4 g/day for males and females respectively, is in line with the results from Potgieter *et al.* (2011), who found habitual alcohol intakes of 14.9 g/day and 2.9 g/day for males and females respectively (71).

Micronutrients

Two micronutrients, namely calcium and iron are of particular relevance in this study population. The micronutrient content of supplements were not quantified, therefore, it should be noted that, in some cases, calcium and iron intake may even be higher due to the use of supplements.

Calcium and the calcium: protein ratio is important for bone health, while sufficient dietary iron intake is important to reduce the risk for iron deficiency anaemia which is associated with decreased aerobic capacity and could thus influence sporting performance.

Calcium is essential for numerous processes in the body, such as the maintenance, growth and repair of bone tissue; the maintenance of blood calcium levels; muscle contraction; nerve conduction and blood clotting (183). A decrease in calcium intake can lead to an increased risk for developing low BMD, osteopenia or osteoporosis (183). As these parameters were studied in the current study population, it was necessary to evaluate the subjects' calcium intake as well. When grouping males and females together, the subjects

consumed adequate amounts of calcium (1000-1500 mg/day), when compared to DRIs of 1000-1200 mg/day (176). When males and females were evaluated independently, the males had a lower calcium intake, as mean calcium levels in this group were below 1000 mg/day. These levels were however, still above 67% of the DRI and therefore adequate. The females, had a mean calcium intake of 1200-2200 mg/day, which is also adequate when compared to the DRI of 1000-1200 mg/day (176). These results are slightly different from those found in 2011 by Potgieter (1250.4 mg/day and 968.1 mg/day for males and females respectively) (71). Guezennec *et al.* (1998) found that in 10 737 school children, college students, military personnel and athletes registered in sports federations (age range 7-50 years), the mean calcium intake was 1242 ± 842 mg/day but that 50% of the population consumed < 1000 mg calcium per day (250).

When evaluating bone health, it is also fitting to monitor or evaluate the calcium:protein ratio, which should be 20 mg of calcium for each gram of protein consumed (i.e. 20:1) (204). In the current study, the subjects had a higher than recommended protein intake but an adequate calcium intake. This led to a low calcium:protein ratio of 10:1 in all subjects, 9:1 for males and 10-12:1 for females.

This low calcium:protein ratio is concerning as an increased animal protein intake is associated with increased urinary calcium excretion, thus if adequate dietary calcium is not ingested sufficient amounts of dietary calcium will not be available for bodily functions such as bone mineralization (251). On the other hand, intake of sufficient amounts of dietary protein is also important to provide substrate for bone matrix as well as to stimulate insulin-like growth factor-1 (IGF-1) needed for osteoblast-mediated bone turnover (252) and therefore, both nutrients should be ingested in the correct amounts. Athletes should be educated on increasing dietary calcium intake further as their protein intake increases, as many already have low bone mass.

Sufficient iron intake is also important, especially for endurance athletes, as iron is needed to form haemoglobin and myoglobin, as well as enzymes important for energy production (183, 253). The oxygen-carrying capacity of iron is particularly important for endurance athletes, and for normal functioning of the nervous and immune systems. Iron deficiency is common in athletes, especially in females with a low energy intake. Such a deficiency can severely reduce muscle function and exercise capacity (183, 253). Haymes *et al.* (1989) found that in 11 female distance runners, 12 sprinters and 11 moderately active females that low levels of ferritin is more common amongs distance runners than sprinters (254).

In the present study, the subjects had a total iron intake of 17 mg/day; the male group had a mean intake of 20 mg/day and the female group a mean intake of 14 mg/day. These daily intakes are sufficient when compared to the DRI's for iron (6-8 mg/day for females and 8-18 mg/day for males) and is in accordance with previous results from Potgieter *et al.* (2011) on triathletes (21.5 mg/day and 14.9 mg/day for males and females respectively) (71).

It was expected that the daily iron intake for both male and female subjects would be adequate, as the subjects' protein intake was sufficient, if not abundant, in the two days leading up to T1 and T2. Protein-rich foods are good sources of especially heme-iron, which has a high bioavailability (255) and the athletes in the present study had an adequate protein intake.

The subject's diets were characterised by protein being one of the most widely-ingested food groups, with chicken, eggs, red meat, peanut butter, low-fat milk and yogurt, and tuna, all good sources of protein were amongst the top 15 most consumed food items.

It has been reported that an iron deficiency is common among female runners. Reasons why iron deficiency is more prevalent among female runners include that they often consume an inadequate dietary intake of protein and vitamin C, a high dietary intake of fibre, which interferes with iron absorption and the increased losses through menses and losses through sweat (256), which is not the case in the current study population. The females consumed adequate if not slightly elevated amounts of protein, normal intakes of dietary fibre, had normal haemoglobin levels and the females also reported mostly normal menstrual function, thereby reducing their risk for iron deficiency anaemia.

Characteristics of dietary intake two days before T1 and T2

It is also important to consider the characteristics of the dietary intake in the two days before T1 and T2 as not only the total energy- and nutrient content is important, but also consuming the right amount of foods from a variety of food groups. The subjects, on average, ate 6-7 meals per day, as per prudent dietary and food-based dietary guidelines. Although the CHO intake was lower than the recommendations for athletes, the most widely-consumed food groups were starch and protein, with the top 15 food choices including oats, brown bread, rusks, fruit, pasta, pizza, red meat, chicken, tuna, eggs, and low-fat milk or yogurt. Reasons why the athletes had a low CHO intake, may have been due to small portion sizes, to keep body weight low before the race, to avoid gastrointestinal discomfort before the race or because of insufficient knowledge regarding the benefits of increasing CHO intake in the days leading up to a race.

It was also interesting to note that the drink consumed most often by the subjects was rooibos tea. This may have occurred due to caffeine/coffee abstinence being a requirement of the study. It was evident from the food groups chosen, that the subjects enjoy a diet low in fat and high in protein, with the exception of pizza, which was eaten quite often. Lastly, the CHO foods chosen were mostly high in fibre, which is in accordance with prudent guidelines (257).

5.4.4.2 Pre-event dietary intake before T1 and T2

It is recommended that the CHO intake for a pre-event meal be between 1-2 g/kg BW, 3-4 hours before the event (174) or 1-4 g/kg BW, 1-4 hours before the event (169). Low to moderate glycaemic index (GI) foods may be beneficial as part of a pre-event meal, if athletes do not consume CHO during the exercise. However, the effect of the pre-event GI is diminished as soon as CHO are ingested during subsequent exercise. Individual tolerance to the type and amount of CHO and preference of the type of CHO is important in order to ensure gastrointestinal comfort and optimal exercise performance (168).

According to the latest ISSN recommendations, protein should be added to the pre-event meal. The recommendation is to include 0.15-0.25 g/kg BW protein with the recommended 1-2 g/kg BW CHO, 3-4 hours before an event (174).

Most of the subjects (85%) ate a pre-event meal the morning of the triathlons. The most commonly-consumed foods were bananas, oats (50-100g) and brown bread (1-2 slices) with peanut butter. This pre-event meal was sufficient with regards to protein content (0.2 g/kg BW and 0.1 g/kg BW for males and females respectively), but the CHO content was inadequate (< 1 g/kg BW for all subjects, and the male and female groups). The dietary CHO and protein intake did not differ in the pre-event meal before T1 and T2. However, as expected, the CHO content of the pre-event meal influenced the overall time to complete T2 in all subjects. It is thus evident that a higher CHO content in the pre-event meal leads to a decreased overall time to complete T2. The reason why there may be differences in correlations between CHO intake and performance in T1 and T2, despite the fact that the mean CHO intake before T1 and T2 is similar is due to the fact that mean intakes are reported and inter-subject variability between T1 and T2 may exist.

The average CHO intake of T1 and T2 of the pre-event meal was much lower compared to studies on high-level triathletes. Cox *et al.* (2010) analysed the pre-event and during event dietary intake of 44 male and 18 female triathletes. The authors found that the male athletes

consume on average 2.9 g/kg BW and 3.3 g/kg BW CHO in the pre-event meal for males and females respectively (258). This is much higher compared to our current study group (0.8 g/kg BW and 0.5 g/kg BW for males and females respectively). Cox *et al.* (2010) also reported pre-event protein intake to be 0.5 g/kg BW for males and females (258), which is much higher than the current study group. Fat intake for the pre-event meal as determined by Cox *et al.* (2010) was 0.3 g/kg BW and 0.2 g/kg BW for males and females (258), whereas our current study group had slightly lower fat intake before the event (0.2 g/kg BW for males and 0.1 g/kg BW for females). Comparing our results with the study by Cox *et al.* (2010), it is evident that the elite level triathletes studied had a more substantial pre-event meal compared to our study group. A possible explanation for this may be that our athletes were asked to bring their pre-event meal to the race, and consume it after the baseline blood samples were taken.

5.4.4.3 Dietary intake during T1 and T2

Muscle fatigue and low blood glucose levels are common complaints during endurance exercise. These complications can inhibit exercise performance. Therefore, optimal CHO intake during endurance events is pivotal in sustaining desired sporting performance (168). McGawley *et al.* (2012) investigated the effect of CHO supplementation compared to placebo supplementation during a simulated Olympic-distance triathlon in six male and four female amateur triathletes. The authors found that CHO supplementation improved triathlon performance and that supplementing with CHO during the cycle leg of an Olympic-distance triathlon, significantly improves subsequent 10 km run performance (259). Therefore, the recommended CHO intake during events lasting longer than 60 minutes is 0.7 g/kg BW or 30-60 g/hour (174) and 30-60 g/hour for events lasting 1-2.5 hours (169). The ADA and ACSM recommend that CHO be ingested at a rate of 30-60 g/hour (183).

Only 62% of the subjects in the current study ingested a food or drink during the triathlons. This is low, considering the importance of ingesting CHO during endurance events. Reasons why the triathletes did not take in any food or drink during the triathlons could have included difficulty to carry the drinks, especially during the swim and run sections (however, during transition and the cycle it would have been easier), availability of drinks (the researcher only provided water during the race, the athletes consumed their own food/drink during the triathlons) or insufficient knowledge regarding the importance of consuming CHO during an event. Most of these subjects ingested some form of CHO-electrolyte solution, such as Energade® (8.0 g CHO/100 ml), PVM Octane® (83.1 g CHO / 100 g) or 32 GI® (94.2 g CHO / 100 g). Subjects also chose PVM® energy bars (1-2 bars per race) (60.9 g CHO / 100 g) and

GU[®] energy gels (3-4 gels per race) (62.5 g CHO / 100 g) as food consumed during the triathlons. When comparing the dietary intake of the study group to the recommendations mentioned before, it is evident that the athletes who were taking CHO during the event were consuming sufficient amounts (> 0.7 g/kg BW). The CHO intake for the male group differed between T1 and T2; however, it did not have an effect on the overall time to complete the events in all subjects, males or females.

The carbohydrate intake ingested during the event in our current study group, compares favourably to the results reported by Cox *et al.* (2010) in terms of the CHO content ingested during the race. Although, in our study we had less subjects ingesting CHO during the event, the amount reported were in line with recommendations and higher than those reported by Cox *et al.* (2010). In the present study the CHO intake during the event was 87 g and 82 g for males and females respectively, compared to 48 g and 49 g for males and females respectively as reported by Cox *et al.* (2010) (258).

As discussed in the literature review, the addition of protein to carbohydrate (CHO: Protein ratio of 3-4:1) during exercise has proved beneficial in terms of improving endurance performance, enhancing muscle glycogen stores, decreasing damage to muscle fibres and enabling better adaptations to training (174, 177, 179, 180). The current study population had a very high CHO:protein ratio, meaning they were not adding protein to the carbohydrate-electrolyte solution consumed during exercise or ingesting any type of protein during exercise. This is, however, still an experimental practice because there are studies showing no improvement in performance with the addition of protein (181, 182). The researcher would therefore not at this stage recommend athletes to add protein to their carbohydrate-electrolyte solutions during exercise. Although, by doing so won't cause any harm or impede exercise performance.

The results obtained from the dietary intake data of the athletes and the practical application emanating from these results are discussed in the following paragraph.

The subjects had a diet low in energy, $_{est}EA$ and CHO and should be educated on the importance of increasing their energy and CHO intake in the days leading up to a race as this could significantly improve their triathlon performance. The $_{est}EA$ should have been higher in these two days, as the subjects were tapering for the race and therefore, heavy training days would further decrease $_{est}EA$ in the present study population. The CHO intake neither reached recommendations for habitual CHO intake nor for CHO loading which is recommended in the days leading up to a race. This has a direct effect on triathlon

performance as CHO loading strategies have been proven in literature to be beneficial in terms of sport/exercise performance. All other nutrients, including protein fat, iron and calcium intake was adequate. The calcium:protein ratio was low for all, suggesting that they are not taking in enough calcium for the amount of protein they are taking in and this could possibly negatively affect bone health.

The pre-event meal of the subjects was low in CHO. As expected and in accordance with literature, the low CHO intake negatively influenced the overall time to complete the triathlons. Only 2/3 of the athletes consumed a carbohydrate-electrolyte solution during the triathlon. Athletes should be educated to include a carbohydrate-electrolyte solution during sporting events lasting longer than 90 minutes as it has been proven to be beneficial for sport performance. Of these 62% drinking carbohydrate-electrolyte solutions, the CHO content was adequate.

Dolan *et al.* (2011) reported that only 19.7% of 401 triathlete's surveyed regarding their training, nutrition, and mental preparation consulted with nutrition professionals and that > 55% of these triathletes consulted with triathlon coaches (243). These statistics, coupled with the dietary inadequacy found in the current study emphasizes the need for athletes, and the triathletes in the present study to consult with nutrition professionals in order to obtain sufficient and evidence-based knowledge regarding nutrition and triathlon performance.

5.4.5 Body composition and bone mineral density

5.4.5.1 Bone densitometry

The role of exercise in bone health

The relationship between physical activity and bone densitometry is complex in nature. Although the benefits of regular physical activity on bone mineral density (BMD) have been shown (260), such activity does not always seem to have a beneficial effect on bone metabolism (261). Bone mineral density can be reduced during high level training and even in some rigorous recreational activity (261). Nichols *et al.* (2007) also described that impact sports may improve BMD, but non-impact sports such as swimming and cycling have no benefit in terms of BMD (262). Important factors when considering the positive or negative effects of sport or physical activity on BMD includes the impact force that causes compression or deflection of bones, hormonal regulation, the type and intensity of exercise, gender and age (261).

Guillaume *et al.* (2011) confirmed that cycling has no beneficial effect on BMD and found that many cyclists had decreased BMD when compared to non-active controls. These authors attributed the decreased BMD to the biomechanics of cycling, which does not inflict loading on the bones and therefore does not increase BMD (263). Other studies found lower BMD in runners and swimmers compared to judoists, but no difference between runners and swimmers (264). Hinrichs *et al.* (2010) reported lower BMD, especially of the lumbar spine, in cyclists, triathletes and runners, but intermediate BMD of the femur in runners and triathletes. The authors attributed this to the repetitive impact of running on the femoral neck. The authors also stated that endurance athletes, in particular runners, can have increased bone loss due to hormonal imbalances and nutritional inadequacy (261).

In the present study, 18% (2/11) of males < 50 years had low BMD and 33% (1/3) of males > 50 years presented with osteopenia of the anterior-posterior spine and a high prevalence of low BMD (72% (5/7)) and osteopenia (40% (2/5)) was found in the pre –and post-menopausal females respectively. The whole body classification of the females also showed that 14% (1/7) (pre-menopausal) and 20% (1/5) (post-menopausal) were osteoporotic, according to the WHO and ACSM criteria for the two groups respectively. This finding is significant in terms of the risk factors for developing osteoporosis, especially in light of the low $_{est}EA$ and the nature of training and competing in triathlons. Nichols *et al.* (2007) reported that the positive effect of physical activity on BMD can be reduced by nutritional status, and especially by low $_{est}EA$ (262).

The role of $_{est}EA$ in bone health

Low $_{est}EA$ as described in point 5.2.2.1, can negatively impact on bone health and development. When $_{est}EA$ is < 30 kcal/kg FFM, it can indirectly influence bone health by influencing bone turnover and leading to amenorrhea in females. Amenorrhea in turn, reduces circulating levels of estrogen, a hormone which is important for bone formation. It is therefore recommended to increase energy intake to compensate for the increased energy expenditure during training in order to improve $_{est}EA$ (206).

Increased physical activity can decrease BMD and increase bone loss due to athletes presenting with a negative energy balance (265). A reduced BMI can lead to reduced bone formation and disturbances in endocrine function. Nutrition is important, as it provides the substrate for synthesis of bone tissue as well as increased levels of hormones that are important for bone health (265). These endocrine disturbances include the attenuation of insulin release and increased levels of cortisol, catecholamines, glucagon and growth

hormone (265). The latter hormones are responsible for enhancing fatty acid oxidation during times of strenuous or prolonged physical activity when carbohydrate is preserved for tissues such as the brain (265). During prolonged exercise, muscle and liver glycogen stores become depleted and proteolysis occur in order to provide substrate for gluconeogenesis (265). This is present when an athlete exercises in an energy deficient state. When this action is repeated regularly and sustained during heavy training, endocrine changes occur. An increased cortisol level decreases osteoblast function and increases osteoclast function, whereas a reduced insulin-like growth factor-1 (IGF-1) reduces osteoblast function as well as synthesis of bone collagen (265). These changes can also lead to a negative nitrogen balance, which disrupts skeletal integrity by reducing muscle mass and strength (265).

Miller *et al.* (2012) reported findings regarding energy deficiency, menstrual disturbances and low BMD on 191 physically active Australian females (18-40 years). The authors concluded that very few (< 10%) of these women have the knowledge regarding the negative effects of low $_{est}EA$ or energy deficiency and subsequent menstrual dysfunction on bone health (266). Therefore, education programs are essential to ensure that athletes take the necessary steps to prevent this negative effect on bone health. Ihle *et al.* (2004) evaluated the dose-response relationship between $_{est}EA$ and bone turnover in 29 healthy, young exercising women and found that bone resorption is increased when the $_{est}EA$ is < 20 kCal/kg FFM (247). Barrack *et al.* (2010) found in 39 female adolescent cross country runners that an increased bone turnover was associated with energy deficiency (267).

The $_{est}EA$ in the current study was calculated for two days prior to the race, while the subjects were tapering. Therefore, it is safe to say that the $_{est}EA$ would be even lower in this group on hard training days. Although there was no statistically significant correlation found between $_{est}EA$ and BMD in the current study population, the low $_{est}EA$, coupled with the high prevalence of low BMD and osteopenia is worrying. It should be recommended to the athletes in the present study to sustain energy balance by specifically increasing carbohydrate intake to replace glycogen stores and to maintain nitrogen balance, and where necessary to increase body weight.

The role of menstrual function in bone health

The high prevalence of low BMD, osteopenia and even osteoporosis in the female group of the present study, warrants the discussion of menstrual function and menopause in relation to bone health. Estrogen is an important hormone in terms of bone health. Decreased levels of estrogen can increase bone turnover and bone resorption (259).

In the present study, a high prevalence of low BMD (72% (5/7)) and osteopenia (40% (2/5)) was found in the pre –and post-menopausal females respectively. The whole body classification of the females also showed that 14% (1/7) (pre-menopausal) and 20% (1/5) (post-menopausal) was classified as osteoporotic.

In pre-menopausal women, energy deficiency is associated with hypoestrogenism (268). An estrogen deficiency combined with energy deficiency increases bone loss and resorption and decreases bone formation (268). Therefore, the energy deficiency is the primary stimulus (259).

Being female in itself is a risk factor for the development of osteoporosis due to the fact that females are in general smaller and thinner compared to males and because menopause has been shown to be an independent risk factor for developing osteopenia / osteoporosis due to the significant decrease in estrogen production that occurs during the first 4-8 years after menopause. Estrogen, which is produced by the ovaries, has a protective effect on bone. Menopause is typically reached between the ages of 45-55 (269-271) and therefore, the prevalence of osteoporosis is higher in women over the age of 50 as the hormonal influence of estrogen on bone health decreases after menopause (272). The consequence of which are changes in bone structure, quality and density, leading to fractures and an increased risk in morbidity and mortality (272). A systematic review conducted by Wallace and Cumming (2000) reported that in post-menopausal women, the BMD of the lumbar spine was positively influenced by impact and non-impact exercise (263).

Other factors influencing bone health

As described in the previous point, the incidence of primary osteoporosis may be higher in females than in males, due to normal bone loss with age, coupled by bone loss in females after menopause. Several risk factors (271) for the development of osteoporosis is prevalent in the current study population, including, but not limited to gender (female), race (Caucasian), age (some females are > 40 years), menopause (five females are post-menopausal, which leads to decreased oestrogen levels and bone loss), excessive exercise (especially non-weight bearing exercise such as swimming, cycling and running), a diet high in protein (>75 g /day) with a low calcium:protein ratio, low energy intake, low _{est}EA and a habitual high caffeine intake (caffeine intake of > 1 000 mg/day can lead to increased urinary calcium excretion and a negative calcium balance). Other risk factors not present in the current study population include a high fibre intake (subjects had a normal fibre intake) and

illness or use of medication (subjects did not report any illness or use of medication that might influence bone health). Risk factors not measured/reported in the current research study, include a family history of osteoporosis, hormone replacement therapy, excessive alcohol use, smoking, high dietary intake of sodium, vitamin A, aluminium and phosphorus, low exposure of, or dietary intake of vitamin D, magnesium and fluoride.

In the present study, the researcher also found a significant positive correlation between height and BMD, with an increased height being associated with an increased BMD. This could possibly be due to the excess weight beared by the skelet, as small body size is a risk factor for developing osteoporosis. This link warrants further investigation in terms of the relationship between height, frame size and BMD.

5.4.5.2 Body composition and anthropometry

Measuring body composition is a valuable tool in assessing and enhancing sporting performance. Changes in body composition can be used to identify the type of training and how much training is needed to optimize body composition for a specific sport or exercise program. Seasonal variations exist with regard to body composition measurements and values should be interpreted according to training programs (187). The present study was conducted directly after the competitive season and therefore it was assumed that the athletes would have a better body composition range than might be expected out of season. Anthropometry, in particular the determination of the body mass index (BMI) using height and weight measurements, has limited application in sport performance. Although the BMI has been shown to correlate well with estimates of fat mass when the BMI is within the normal ranges (18.4-24.9 kg/m²) and in non-athletic populations, it can significantly under- or over-estimate fatness at the extremities of the BMI ranges. It is known, for example that using the BMI, a very muscular person can be classified as being overweight or a very lean person as underweight (although not malnourished) (187). This is seen in the current study as well. Although the total subject group, males and females had a normal BMI, the males had a higher BMI compared to females, even though they had lower percentage body fat. Therefore, it is important to determine all components of body composition in athletes, not only to determine the influence of training programs, but also to determine the influence of fatness on exercise performance.

This is of particular importance in female athletes, as a body fat percentage below the recommended range can influence not only sporting performance, but also sexual maturation and fertility (187, 273). It has also been reported that eumenorrheic and amenorrheic athletes have similar body fat percentages, suggesting that the main risk factor

for menstrual dysfunction is energy deficit, and not necessarily the percentage of body fat or body composition (274). An increase in energy intake, without reducing exercise volume, restores reproductive function, suggesting that energy intake, rather than training volume, is important when evaluating sexual maturation and fertility (274). Low energy intakes are not only prevalent in female athlete populations, but in male athletes as well. In the latter, lower fatness, BMD and rapid weight loss can lead to decreased testosterone levels, influencing overall health and athletic performance (275).

The percentage body fat in the current study population was interpreted as appropriate for triathletes, according to reference values from the ADA and ACSM (i.e. 6-15% for male and female triathletes). The males in the current subject group had a mean percentage body fat of 10-11%, which is within the abovementioned recommended range for triathletes. The females in the current study group, however, had a higher than recommended percentage body fat (19%) when compared to the ADA recommendations of 6-15% (207, 276).

Although the ideal percentage body fat for athletes and triathletes specifically differs in the literature, there appears to be a general trend toward optimizing body composition to optimize triathlon performance. The ideal percentage body fat has been described as between 6-10% for male and 11-18% for female triathletes (277). According to Worme *et al.* (1990), who did a study on 21 female and 50 male recreational triathletes, the percentage body fat was 15% and 24% for men and women respectively (244). The latter agrees with findings in the previous study by the researcher on triathletes residing in the Western Cape, where the percentage body fat was found to be 13% for men and 21% for women (71). A study done on eight female and ten male athletes competing in the Ironman triathlon in New Zealand found the percentage body fat to be 15% and 22% for men and women respectively (278).

To summarize; male triathletes have a percentage body fat within the recommended range, while female triathletes have levels similar to those of sedentary women. A positive correlation was found between height and BMD. Although there were no statistical significant correlations between BMI, body weight and BMD in the present study, the BMI of the athletes fell within the normal range, which might have a positive effect in terms of protection against the development of osteoporosis as a decreased BMI or body weight are also seen as risk factors for developing osteoporosis. This is of relevance in our study population as there was a high prevalence of low BMD and osteopenia in the male and female groups. Together with other risk factors, such as low _{est}EA, low energy intake, low calcium:protein

ratio, menopause, pre-disposition due to ethnicity and excessive non-weight bearing exercise, these athletes are at an increased risk for developing osteoporosis.

5.4.6 Training two days before race day

5.4.6.1 Usual training habits/regime

The current study population can be described as provincial-level triathletes. All subjects were either part of the 2010 or 2011 Western Province Triathlon team. The usual duration of training per week (± 13 hours) is in accordance with reported literature on senior national Olympic-distance triathletes from Great Britain, who trained for approximately 15 hours per week (279). The triathletes in the current study trained on average 1-2 times per day, averaging 1-2 hours per training session.

The average number of Olympic-distance triathlons completed by the whole subject group, in the preceding year was 4-5, which included amongst others, Western Province trials, Western Province Championships and the South African Championships. Some of the subjects also competed at the World Triathlon Championships. The Olympic-distance personal best finishing time ranged from 2 hours 20 minutes for males to 2 hours 30 minutes for females, which is in keeping with finishing times of amateur-level male and female triathletes (2 hours 20 minutes) reported by Jeukendrup *et al.* (2005) (121).

5.4.6.2 Training characteristics before triathlon 1 and triathlon 2

Training characteristics were also determined for the two days leading up to T1 and T2. This was important to assess adherence to the research protocol and to ensure that the training characteristics were the same before each event. It is recommended that athletes taper (i.e. decrease the amount and frequency of training, not the intensity of training) in the two days leading up to a race. The subjects adhered to the study protocol and the only training characteristic that differed between the two days preceding T1 and T2 was the running exertion in males, which was rated slightly, but statistically significantly higher before T2. However, this did not influence the overall time to complete either T1 or T2.

In summarising the training characteristics of the subjects; the athletes spent ± 13 hours per week training, including swimming, cycling, running and other exercise such as rowing or going to the gym. This classifies them as being amateur, age group or provincial-level triathletes. The training before T1 and T2 did not differ significantly and did not influence their overall time to complete T1 or T2.

5.4.7 Caffeine withdrawal

The subjects were asked to abstain from caffeine and caffeine-containing products for two weeks (14 days) before T1 and T2. Due to the abstinence of caffeine in the diet, caffeine withdrawal was expected in subjects who habitually use > 300 mg caffeine per day (our study population had a habitual caffeine intake of ± 400 mg /day). Subjects were asked to complete a questionnaire (Appendix 3.13) before T1 to describe any possible caffeine withdrawal symptoms experienced in the two weeks prior to T1. Expected symptoms of caffeine withdrawal included headaches, fatigue, lethargy and flu-like symptoms, weariness, apathy, weakness, drowsiness, decreased motor behaviour, increased heart rate, increased muscle tension, tremor, nausea and vomiting (26, 41, 57, 68, 100, 280-284).

These symptoms typically commence within 12-24 hours after abstaining from caffeine (57, 68, 285) and peak within 24-48 hours after cessation of caffeine intake. Plasma caffeine levels decrease to baseline (< 1 mg/l) within 4-7 days after caffeine abstinence (57, 69). As expected, all these withdrawal symptoms can have the potential to significantly inhibit exercise performance, particularly in the 2-4 days after the commencement of caffeine withdrawal. As soon as caffeine intake is resumed, however, the symptoms subside. It is suggested that athletes avoid caffeine and caffeine-containing products for at least one week before competitions, to allow any caffeine withdrawal symptoms to have passed (69). Withdrawal symptoms have been reported in subjects consuming as little as 129 mg per day (57, 283, 284).

In the current study group, almost half of the subjects (46%) experienced headaches in the two weeks before T1. Frequent and severe headaches are caused by vasodilation of cerebral blood vessels due to caffeine abstinence (69). Other symptoms, such as fatigue, lethargy and flu-like symptoms were present in approximately a third of subjects. Only headaches experienced during the two weeks prior to T1 influenced the overall time to complete T1; all subjects, and the male and female groups that did not experience headaches during this time demonstrated a significantly shorter overall time to complete this triathlon.

5.4.8 Side-effects of caffeine supplementation

The subjects were asked to complete a questionnaire on the side-effects of caffeine that may have been experienced. The caffeine group experienced statistically significantly more shakiness, heart palpitations and gastrointestinal disturbances compared to the placebo group. However, when these side effects were entered as covariates to determine the effect

of these on the overall time to complete the triathlons, only heart palpitations influenced the overall time to complete the triathlon. However, this association was not due to caffeine supplementation, as the overall time to complete the triathlon was longer in the placebo group who experienced heart palpitations, compared to the caffeine group.

Therefore, caffeine supplementation in the present study increased plasma caffeine levels to peak plasma caffeine levels as expected. We can conclude from this that using 70% microencapsulated caffeine is effective in elevating plasma caffeine levels sufficiently to have an ergogenic effect. Caffeine withdrawal was experienced by the subjects during the first 14 days of caffeine abstinence; the most prevalent withdrawal symptoms experienced included headaches, fatigue, lethargy and flu-like symptoms. The authors suggests that athletes who plan on using caffeine as an ergogenic aid, abstain from caffeine containing products at least 14 days prior to their race, to allow sufficient time for withdrawal symptoms to pass. Some of the athletes experienced symptoms such as heart palpitations, shakiness and GIT disturbances. Although these are symptoms recognized in the literature as side effects of caffeine supplementation, they occurred irrespective of caffeine supplementation in the present study and were not association with the overall time to complete the triathlons.

5.4.9 Hydration status and changes in plasma volume

Albumin constitutes almost 60% of plasma proteins. It is produced by the liver and exerts osmotic pressure to maintain water balance between blood and tissues (5). Serum albumin levels in the present study were determined to detect any changes in plasma volume that might occur due to exercise and to adjust other biochemical parameters accordingly. Serum albumin levels measured at baseline, during transition (cycle → run) and at the finish line fell within the recommended reference range (35-50 g/l), with the exception of the male group's serum albumin measured during transition (cycle to run), which was elevated beyond the normal reference value. The serum albumin levels for all subjects, and the male and female groups were elevated during transition (cycle → run), but returned to baseline values when measured again at the finish line.

The only parameter measured during transition (cycle → run), was plasma caffeine levels. Plasma caffeine does not need to be corrected for albumin values. All the other parameters that could potentially be influenced by changes in plasma volume, such as the full blood count), were only measured at baseline and at the finish line. There were no significant differences in baseline and finish line serum albumin levels in all subjects, and the male and female groups, irrespective of whether caffeine supplementation was given or not.

To conclude, serum albumin levels did not differ significantly from baseline to finish line in the present study. Therefore it was not needed to adjust any blood values as caffeine supplementation had no effect on hydration status and the athletes maintained plasma volume before and after the triathlons.

General health, energy- and nutrient intake two days before as well as dietary strategies followed on race day, body composition and bone mineral density, training two days before race day, side-effects of caffeine withdrawal, side effects of caffeine supplementation and hydration status and plasma volume may influence Olympic-distance triathlon performance.

CHAPTER 6: CONCLUSION

6.1 Summary of findings

The main aims of this study were to i) investigate the performance-enhancing or ergogenic effect of caffeine supplementation during a real-life triathlon competition; ii) evaluate several parameters that could in part explain why caffeine supplementation is ergogenic, iii) investigate possible factors influencing the ergogenicity of caffeine supplementation and iv) investigate possible confounding factors influencing Olympic-distance triathlon performance.

To our knowledge, this is the first study evaluating these factors in relation to caffeine supplementation and triathlon competition.

Caffeine supplementation improved Olympic-distance triathlon performance. This effect was seen in both males and females. The greatest effect was seen on the overall time to complete the triathlon, as well as the swim section of the triathlon. There were no statistically significant differences in RPE values or mood state between the caffeine and placebo trials, although a trend was observed with regard to lower RPE values in the caffeine compared to placebo trial.

Caffeine supplementation made no difference to the markers of endocrine-stress response, except for cortisol, which increased beyond that of the effect observed from endurance exercise in the caffeine, compared to placebo group. Other markers (testosterone, prolactin and dehydroepiandrosterone sulphate) displayed gender differences as expected, but no differences were observed in the caffeine and placebo groups. Exercise-induced inflammation or infection was more pronounced when receiving caffeine supplementation, as seen with the elevated levels of white blood cell and lymphocyte counts in the caffeine compared to the placebo groups. Exercise is an independent stressor and influenced the other components of the full blood count by increasing neutrophils, monocytes, basophils and platelet counts and by decreasing eosinophils, red blood cells and haematocrit, with no change in haemoglobin levels. Caffeine also facilitated greater lactate production. The mechanism by which caffeine elicits its ergogenic effect is not clear from the current study's results, although it appears that the greatest effect of caffeine supplementation is due to its direct effect on the central (antagonism of adenosine receptors) and indirect effect on the autonomic nervous system (increased cortisol levels).

The self-reported phase of the menstrual cycle, menopause status or oral contraceptive use had no statistically significant effect on caffeine metabolism or the overall time to complete the triathlons. Clinically relevant findings were that faster performance times were achieved when the women were in the follicular phase of the menstrual cycle whilst receiving caffeine

supplementation. Post-menopausal women completed the triathlons on average slower than pre-menopausal women, irrespective of caffeine supplementation. Upon genetic analysis, it was evident that in this small subject group, *CYP1A2* gene polymorphism did not influence caffeine supplementation's effect on triathlon performance significantly.

The subjects had a high habitual caffeine intake (± 400 mg/day). The athletes had a good medical history. The dietary intake (including supplement use) two days before T1 and T2 did not differ. The subjects had a diet low in energy, ^{est}EA and CHO, with sufficient protein and fat intake. The subjects had sufficient iron and calcium intake, but a low calcium:protein ratio. This, together with other risk factors, such as a high prevalence of low bone mineral density and osteopenia, menopause, pre-disposition due to ethnicity and excessive non-weight bearing exercise, places this group at risk for developing osteoporosis. The pre-event meal of the subjects contained sufficient protein, although it was low in carbohydrates. Only 2/3 of the athletes consumed a carbohydrate-electrolyte solution during the triathlon. Of these, the CHO content during the event was adequate. The athletes have a percentage body fat within the recommended range for healthy individuals, and spent on average ± 13 hours per week training.

Caffeine withdrawal symptoms, such as headaches, fatigue, lethargy and flu-like symptoms were experienced by the subjects, but 14 days of caffeine abstinence allowed sufficient time for these symptoms to pass and not affect triathlon performance. Side-effects of caffeine, such as heart palpitations, shakiness and gastrointestinal tract disturbances were observed, but did not influence triathlon performance.

Serum albumin levels did not differ significantly from baseline to finish line in the present study. Therefore it was not needed to adjust any blood values as caffeine supplementation had no effect on hydration status and the athletes maintained plasma volume from before to after the triathlons.

6.2 Conclusions

This research study demonstrated that double-blind, randomized crossover controlled clinical field trials and not only laboratory experiments are necessary to establish the real effect of caffeine supplementation on triathlon performance. The researcher also provided an assessment of several parameters that could in part explain the ergogenicity of caffeine supplementation, factors influencing the ergogenicity of caffeine supplementation as well as factors influencing Olympic-distance triathlon performance. Furthermore, to our knowledge,

this study was the first field-study to determine the effects of caffeine supplementation during an Olympic-distance triathlon on performance in both male and female provincial-level Olympic-distance triathletes.

It can thus be concluded that a field trial and not only laboratory experiments are necessary to establish the real effect of caffeine supplementation on Olympic-distance triathlon performance. The researcher therefore accepts the hypotheses, that i) double-blind, randomized, crossover, controlled, clinical field trials and not only laboratory experiments are necessary to establish the real effect of caffeine supplementation on the performance time, rating of perceived exertion and mood state before, during and after an Olympic-distance triathlon; ii) the endocrine-stress response, oxidative stress and plasma lactate levels could potentially explain the ergogenic effect of caffeine supplementation; iii) lifestyle, gender and genetics may influence the ergogenicity of caffeine supplementation although this remains to be substantiated in a larger cohort; and iv) general health, energy- and nutrient intake, body composition, training status, caffeine withdrawal symptoms and side effects of caffeine supplementation influence Olympic-distance triathlon performance.

6.3 Summary of contributions

This is the first study, to the researcher's knowledge, assessing and evaluating the effect of caffeine supplementation on Olympic-distance triathlon performance. This double-blind, randomized, cross-over, controlled clinical field study evaluated the effect of caffeine supplementation on triathlon performance, rating of perceived exertion and mood state. The study was extremely well-controlled in terms of factors that could potentially influence the ergogenicity of caffeine supplementation, such as lifestyle, gender-related factors (menstrual cycle, menopause and oral contraceptive use) and genetics (*CYP1A2* gene polymorphism) as well as factors that could potentially influence exercise performance, such as general health, energy- and nutrient intake, body composition and bone mineral density, training in the days leading up to a race, side-effects of caffeine withdrawal and supplementation and hydration status. The results further indicated the primary mechanism of action with regard to the ergogenic effect of caffeine supplementation is as a result of direct stimulation of the CNS and indirect stimulation of the autonomic nervous system.

This study can be seen as a benchmark for other field studies. A particular strength of the study is the fact that is a field study and not a controlled laboratory experiment. Variations in the effect of caffeine as an ergogenic aid exist when an athlete is placed in the acute stress situation of an actual race.

A particular strength of the study is the multi-disciplinary nature, combining research in nutrition, physiology, exercise science and genetics.

Differentiating between subjects on a gender basis is important and included in this study.

6.4 Limitations of the current study

The sample size in this study was relatively limited in the context of nutritional sciences and genetic analysis. However, when considering other studies in the field of physiology and exercise science, the sample size compares favourably. The South African triathlete population is fairly small, so that the study sample is representative of the available pool.

Genetic analysis was included for completeness due to suggestions of polymorphisms affecting caffeine metabolism and ultimately exercise performance, in the caffeine-related literature specifically. Our result indicates that there was not a major variation in terms of genetic make-up related to caffeine metabolism in our population. However, this obviously does not exclude the possibility of a statistically significant genetic influence on caffeine metabolism in a more diverse and larger group. This issue should be further investigated in a population of adequate total size (e.g. endurance runners) to allow for proper genetic analytical procedures with sufficient statistical power. Furthermore, haplotype analyses would also have been more effective than single SNP analyses, as an extended haplotype defined by the known SNPs could detect an unknown variant influencing caffeine metabolism (286).

6.5 Recommendation for triathletes with regard to caffeine supplementation

The researcher would recommend the use of caffeine supplementation as an Olympic-distance triathlon performance enhancing supplement. The significant decrease in the time to complete the triathlon when given caffeine supplementation can make the difference between winning a race, achieving a podium position or making the cut-off for selection for provincial or national teams. A recommendation can be made to these athletes to ingest 6 mg/kg body weight caffeine, 45-60 minutes before the start of an Olympic-distance triathlon. The researcher would further recommend that athletes abstain from caffeine containing food and drink for two weeks prior to the planned race, in order to achieve the optimal benefit of caffeine supplementation as well as to ensure sufficient time for caffeine withdrawal symptoms to pass, especially as these athletes are high habitual caffeine consumers.

6.6 Recommendations and future research

The present study was novel in that it had a multidisciplinary approach. The study evaluated the effect of caffeine supplementation on Olympic-distance triathlon performance, while controlling for most factors associated with exercise performance. However, due to the multiple variables studied in three distinct fields (nutrition, physiology and genetics), the same depth cannot be expected when compared with studies conducted in only one of these fields.

Furthermore, in our opinion, the current study provides the best possible field assessment of caffeine's benefits in the context of triathlon specifically. Future studies could include larger sample sizes by picking a different sporting discipline, and focus especially on detecting differences between males and females and to further investigate possible effects of *CYP1A2* gene polymorphism. Other genes (such as *CYP3A4* and *CYP2C8/9*) and epigenetic factors can also be included to provide a more comprehensive analysis of the role that various genes and polymorphisms may play (Figure 2.2).

Studies could also be conducted utilising triathlons of shorter or longer duration, to determine the effect of caffeine supplementation performance in non-Olympic-distance triathlons.


Additional extensions to this study can include a more in depth assessment with regards to the dosage of caffeine supplementation in habitual and non-habitual caffeine consumers and the influence of gender and the menstrual cycle on the ergogenicity of caffeine supplementation in a larger cohort.

APPENDICES


APPENDIX 3.1 ADVERTISEMENT FOR RECRUITMENT OF SUBJECTS

Research Triathlon


Come join in the fun of my PhD research Triathlon(s)



Swim 1.5 km



Cycle 40 km



Run 10 km

I want to see how
CAFFEINE influences
your triathlon
performance!!

All WPTA 2010/11 registered athletes!


Dates: 8 May '11
22 May '11
(You have to do both)

Time: 08:00 am

Venue: TBA


Only space for 40 male & 40 female triathletes so RSVP your space now!

Contact details:
Sunita Potgieter
E-mail:
sunita@sun.ac.za
Telephone:
021 938 9474
Cell phone:
082 335 3650




Prize giving after the second triathlon

	Male	Female
20-39 years	1. R 7 000.00	1. R 7 000.00
	2. R 6 000.00	2. R 6 000.00
	3. R 5 000.00	3. R 5 000.00
	4. R 4 000.00	4. R 4 000.00
	5. R 3 000.00	5. R 3 000.00
40-60 years	1. R 7 000.00	1. R 7 000.00
	2. R 6 000.00	2. R 6 000.00
	3. R 5 000.00	3. R 5 000.00
	4. R 4 000.00	4. R 4 000.00
	5. R 3 000.00	5. R 3 000.00



Research Triathlon

Come join in the fun of my PhD research Triathlon(s)



I want to see how
CAFFEINE influences
your triathlon
performance!!

<p style="text-align: center;">Before Triathlon 1 5 May 2011</p>	<ul style="list-style-type: none"> Sign an Informed consent, indemnity waiver & complete questionnaires Instructions for Triathlon 1 Give a saliva sample Height, weight & body composition measurement Start keeping food record 1 (Friday 6 May – Sunday 8 May 2011)
<p style="text-align: center;">Triathlon 1 8 May 2011</p>	<ul style="list-style-type: none"> Complete questionnaires & Weight measurement Give a blood sample Race briefing for Triathlon 1 Caffeine / Placebo Supplementation Swim 1.5km · Transition 1: complete rating of perceived exertion (RPE) Cycle 40 km · Transition 2 : give a blood sample & complete RPE Run 10 km · Finish line: give a blood sample & complete RPE Hand in food record 1
<p style="text-align: center;">Before Triathlon 2 19 May 2011</p>	<ul style="list-style-type: none"> Sign the indemnity waiver & complete questionnaires Height & weight measurement Instructions for Triathlon 2 Start keeping food record 2 (Friday 20 May – Sunday 22 May 2011)
<p style="text-align: center;">Triathlon 2 22 May 2011</p>	<ul style="list-style-type: none"> Complete questionnaires & Weight measurement Give a blood sample Race briefing for Triathlon 2 Caffeine / Placebo Supplementation Swim 1.5km · Transition 1: complete RPE Cycle 40 km · Transition 2: give a blood sample & complete RPE Run 10 km · Finish line: give a blood sample & complete RPE Hand in food record 2

Prize giving

APPENDIX 3.2 FIELD WORKERS TRAINING STANDARDIZATION SESSION

PhD Research Triathlons

Volunteers info session
15 April 2011

Swim 1.5 km

Cycle 40 km

Run 10 km

The effect of caffeine supplementation on Olympic distance triathlon and triathlon performance in the Western Cape region

Researcher: Dr. Gert van der Merwe - Stellenbosch University

Principal: Dr. C. Smith - Department of Physiological Sciences - Stellenbosch University

Co-supervisor: Dr. J. van der Merwe - Stellenbosch University

Co-supervisor: Prof. J. van der Merwe - Stellenbosch University

Supervisor: Prof. D. G. Hall - Stellenbosch University

Co-supervisor: Stellenbosch University

Office approval: Approved by IRB, PHS, & HSE (2011/04/04)

Motivation

- **M Nutrition**
 - 42% of WPTA consume herbal supplements with/without caffeine daily
 - Only 24 consumed caffeine on a habitual basis
 - Need in WC to assess the use of caffeine supplementation → benefit athletes' sport performance
- **Evident from literature**
 - Abundant evidence of the ergogenic effect of caffeine during endurance exercise
 - Most of these studies include protocols on cycling, running, swimming, rowing & skiing & none on the effect of caffeine supplementation on triathlon performance

Motivation

- **Protocols included in these studies**
 - Measure performance effect with "time to exhaustion" protocols
 - Not accurate reflection of exercising in field
 - Greater coefficient of variation when subjects perform a time to exhaustion protocols, time trial performance
 - Time to exhaustion does not measure true exercise performance benefit of caffeine as no sport requires an athlete to endure more or complete a longer distance than his/her competition
 - Triathlon specifically requires an athlete to complete the set amount of work or distance in the shortest amount of time, i.e. a time trial performance
 - Protocol is exercise for 120-240 minutes at constant sub-maximal intensity, which is not applicable to a race situation either
 - No one races at constant sub-maximal intensity

Motivation

- Given these problems & most research on caffeine supplementation was done in a laboratory setting
- Need for a study conducted in a field setting
- We therefore propose to do a field study, utilising optimal performance assessment tools, to provide more applicable data
- Studying the use of caffeine in the field setting will also give valuable information on the effect thereof on the stress response

Motivation

Mode of supplementation

- Capsules
- Gum, soft drinks & coffee have been used previously
- Limited availability of capsules containing caffeine
- Additional ingredients, such as CHO in the other modes (gum, soft drink, coffee), does not isolate the ergogenic effect of caffeine
- Our research study will examine the effect of microencapsulated caffeine (70%), which is a tasteless white powder with no added ingredients

Motivation

Shortcoming in published literature

- Absence of studies differentiating between subjects and/or results on a gender basis
- Large amount of the existing research on caffeine consumption in sport or exercise has only included male athletes
- Only 10 of the 29 trials included women in the study population, of which 2 studies tested only female subjects and the other 8 trials did not distinguish between male and female in the statistical analysis
- The proposed research study will include male & female triathletes



Motivation

- Multidisciplinary nature
 - Proposed study will both give a more comprehensive assessment of the effect of caffeine on the stress response
 - Include novel ideas such as to examine the prevalence & influence of CYP1A2 gene polymorphism on caffeine metabolism and its effect on sports performance
- Large subject number proposed, since most studies investigating the physiology of exercise have very limited subject number, and thus lower statistical power



Motivation


Available research on the mechanism(s) by which caffeine supplementation has its ergogenic effect is controversial & more research in this field is definitely needed to fully understand & comprehend the mechanism(s) by which caffeine enhances performance as well as the extent of improvement in endurance performance



Background


The main aim of my research is to determine to what extent and through which mechanism(s) caffeine supplementation influences Olympic distance triathletes and triathlon performance

The project has various objectives, which include to determine:



Background

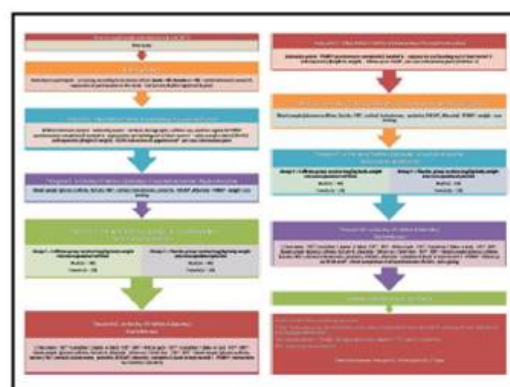
- Dietary intake before and during the two triathlons
- Body composition
- Your mood status before, during and after a triathlon
- Habitual caffeine intake
- How caffeine influences triathlon performance by looking at lactate levels, time to complete the triathlons and your rating of perceived exertion
- Whether caffeine intake before a triathlon influences the body's response to stress by measuring blood samples for full blood count, albumin and hormones like cortisol, testosterone, prolactin, DHEAS
- Whether you metabolize caffeine "fast" or "slow" by analyzing your DNA from a saliva sample



Study Plan

Study design

- Randomized double-blind, placebo-controlled, crossover design
- Each subject will follow the protocol with an interval of 14 days between the two tests





Details of the events:

- Date:** 8 and 22 May 2011
- Venue:** Gordon's Bay Beach
- Time:** 06h15 – 14h30
- Distances covered:** 1.5 km swim, 40 km cycle and 10 km run



Time	Triathlon 1: 8 May 2011	Triathlon 2: 22 May 2011
07h00	Athletes and participants arrive at the event	Athletes and participants arrive at the event
07h00 – 07h45	Baseline blood sample at the Pathcare mobile clinic Bike racking	Baseline blood sample at the Pathcare mobile clinic Bike racking
07h45	Caffeine / placebo supplementation	Caffeine / placebo supplementation
08h30	Start of race – 1.5 km swim	Start of race – 1.5 km swim
08h45 – 09h30	Anticipated finish of the swim Athletes move through transition 1 Start of the 40 km cycle	Anticipated finish of the swim Athletes move through transition 1 Start of the 40 km cycle
09h40 – 11h30	Anticipated finish time of the 40 km cycle Athletes move through transition 2 Athletes move through the Pathcare mobile clinic Start of the 10 km run	Anticipated finish time of the 40 km cycle Athletes move through transition 2 Athletes move through the Pathcare mobile clinic Start of the 10 km run
10h30 – 14h00	Anticipated finish of the 10 km run Athletes move through the Pathcare mobile clinic Finish of the race	Anticipated finish of the 10 km run Athletes move through the Pathcare mobile clinic Finish of the race
14h00 – 14h30		Prize giving



What I need your help with

- 1st years:** Registration, marshals & timing of blood samples
- 2nd years:** Checklist
- 3rd years:** Checklist
- 4th years:** Checklist
- STB students:** Capillary lactate measurements
- All students –** help with clean-up afterwards (☺)
- Pathcare:** Phlebotomists



1st years

- Registration:**
 - 2 volunteers needed to man registration desk where subjects sign indemnity forms
 - Body marking
- Marshals:**
 - Placed around the event map to ensure safety of athletes & map out the course
- Timing of blood samples:**
 - Stopwatches – time how long it takes for phlebotomist to take blood sample during transition 2
 - Give info to "data collectors"



2nd, 3rd & 4th years

- Each will receive 2-3 subjects
- You have to make sure that ALL data for ALL subjects are completed
- Checklist!!!**
- The 2-3 athletes assigned to you is **YOUR responsibility**
- NB! We only have 1 shot to get accurate data!!!**

Data collection checklist:

Subject data collection checklist & data entry form:

Subject reference number: _____

Data collection / task	Value (if applicable)	Yes/No
1. Screen according to inclusion & exclusion criteria		
2. E-mail subject information & instructions leaflet		
3. Explanation of study & informed written consent obtained (1 copy researcher, 1 copy subject)		
4. Completed questionnaires on Survey Monkey		
5. Subject has been caffeine naive since 24 April 2011		
6. Profile of Mood State questionnaire completed (POMS)		
7. Instructions and handing out of food record & small scale for weighing portion sizes given		
8. Buccal swab sample taken		
9. Weight measurement taken, write value:		
10. Height measurement taken, write value:		
11. DEXA scan completed		
12. Pre-race info & race pack given		

Data collection / task:	Value (if applicable)	Yes/No
Week before T1 (2-5 May 2011)		
1. Screen according to inclusion & exclusion criteria		
2. E-mail subject information & instructions leaflet		
3. Explanation of study & informed written consent obtained (1 copy researcher, 1 copy subject)		
4. Completed questionnaires on Survey Monkey		
5. Subject has been caffeine naive since 24 April 2011		
6. Profile of Mood State questionnaire completed (POMS)		
7. Instructions and handing out of food record & small scale for weighing portion sizes given		
8. Buccal swab sample taken		
9. Weight measurement taken, write value:		
10. Height measurement taken, write value:		
11. DEXA scan completed		
12. Pre-race info & race pack given		

Transition 1 (2-5 May 2011)	Value (if applicable)	Yes/No
1. Registration (Indemnity signed, body marking)		
2. Profile of Mood State questionnaire completed (POMS)		
3. Baseline blood sample taken - Fasting, 2 vials for Dept. Physiology		
4. Finger prick for capillary lactate, write value:		
5. Race briefing		
6. Caffeine / placebo supplementation given		
7. Race briefing		
8. Race briefing		
9. Transition 1: Time to complete transition 1		
10. Transition 1: Borg rating of perceived exertion		
11. Cycle 40 km: Time to complete cycle		
12. Transition 2: Time to complete transition 2		
13. Transition 2: Borg rating of perceived exertion		
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Transition 2 (2-5 May 2011)	Value (if applicable)	Yes/No
1. Registration (Indemnity signed, body marking)		
2. Profile of Mood State questionnaire completed (POMS)		
3. Baseline blood sample taken - Fasting, 2 vials for Dept. Physiology		
4. Finger prick for capillary lactate, write value:		
5. Race briefing		
6. Caffeine / placebo supplementation given		
7. Race briefing		
8. Race briefing		
9. Transition 1: Time to complete transition 1		
10. Transition 1: Borg rating of perceived exertion		
11. Cycle 40 km: Time to complete cycle		
12. Transition 2: Time to complete transition 2		
13. Transition 2: Borg rating of perceived exertion		
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Indemnity form

Indemnity form

Subject reference number: _____

I, the undersigned, _____ hereby agree to participate in the trial as part of a research study.

I confirm that my participation in the study and the related activities is voluntary and I understand all the involved risks. The research or any of the participating institutions or individuals do not accept any of their respective employees or persons shall not be liable for any loss, damage, injury or illness of whatsoever nature and I understand that I am not entitled to any compensation or payment for participating in the study.

Signed at: _____ on: _____ at: _____ 2011

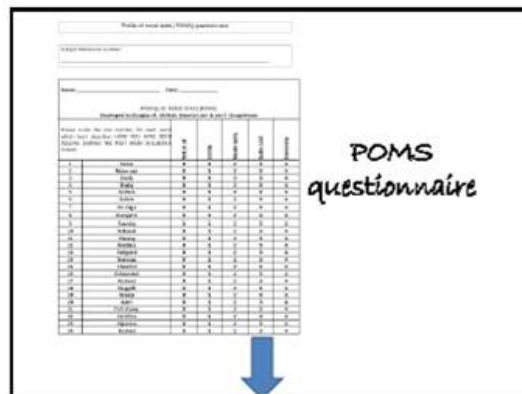
Signature of participant: _____ Place Name: _____


Body Marking

Race / Subject numbers





RACE RULES - GENERAL






Cycle 40 km → Transition 2




TRANSITION 2

Borg scale
Time to complete
Pacecare & NB time to take blood sample
Finger prick

Run 10 km → Finish line




Finish line

Borg scale
Time to complete
Pacecare – final blood sample
Finger prick – 3, 6, 9, 12 & 15 minutes after
POMS questionnaire
Hand in food record



Triathlon 2

- Exactly the same
- Prize giving at the end!



STB Students

- Measure capillary lactate levels & timing (stopwatches)
 - Before race
 - During transition 2
 - After race (at finish line):
 - 3 minutes
 - 6 minutes
 - 9 minutes
 - 12 minutes
 - 15 minutes



What will you receive

- List of duties / Checklist
- Emergency contact list
- Lunch & drink
- T-shirt
- ABSOLUTELY AMAZING RESEARCH EXPERIENCE!!!
- THANK YOU FOR HELPING!!!

APPENDIX 3.3 CHECKLIST

*Subject data collection checklist & data entry form:**Subject reference number:* _____

Data collection / task:	Value (if applicable)	Yes/No
Week before T1 (2-5 May 2011)		
1. Screen according to inclusion & exclusion criteria		
2. E-mail subject information & instructions leaflet		
3. Explanation of study & informed written consent obtained (1 copy researcher, 1 copy subject)		
4. Completed questionnaires on Survey Monkey		
5. Subject has been caffeine naïve since 24 April 2011		
6. Profile of Mood State questionnaire completed (POMS)		
7. Instructions and handing out of food record 1 Small scale for weighing portion sizes given		
8. Buccal swab sample taken		
9. Weight measurement taken, write value:		
10. Height measurement taken, write value:		
11. DEXA scan completed		
12. Pre-race info & race pack given		
Triathlon 1 (8 May 2011)		
13. Registration (indemnity signed, body marking)		
14. Profile of Mood State questionnaire completed (POMS)		
15. Baseline blood sample taken – Pathcare, 1 vacutainer for Dept. Physiology		
16. Finger prick for capillary lactate, write value:		
17. Bike racking		
18. Caffeine / placebo supplementation given		
19. Race briefing		
20. Swim 1.5 km: Time to complete swim:		
21. Transition 1: Time to complete transition 1:		
22. Transition 1: Borg rating of perceived exertion:		
23. Cycle 40 km: Time to complete cycle:		
24. Transition 2: Time to complete transition 2:		
25. Transition 2: Borg rating of perceived exertion:		
26. Transition 2: Blood sample taken, Time to take blood sample:		
27. Transition 2: Finger prick for capillary lactate, write value:		
28. Run 10 km: Time to complete run:		
29. Finish line: Time to complete entire triathlon:		
30. Finish line: Borg rating of perceived exertion:		
31. Finish line: Blood sample taken		
32. Finish line: Finger prick for capillary lactate 3 minutes:		

Data collection / task:	Value (if applicable)	Yes/No
33. Finish line: Finger prick for capillary lactate 6 minutes:		
34. Finish line: Finger prick for capillary lactate 9 minutes:		
35. Finish line: Finger prick for capillary lactate 12 minutes:		
36. Finish line: Finger prick for capillary lactate 15 minutes:		
37. Finish line: Profile of Mood State questionnaire completed (POMS)		
38. Finish line: Hand in food record 1		
Triathlon 2 (22 May 2011)		
1. Registration (indemnity signed, body marking)		
2. Profile of Mood State questionnaire completed (POMS)		
3. Baseline blood sample taken – Pathcare, 1 vacutainer for Dept. Physiology		
4. Finger prick for capillary lactate, write value:		
5. Bike racking		
6. Caffeine / placebo supplementation given		
7. Race briefing		
8. Swim 1.5 km: Time to complete swim:		
9. Transition 1: Time to complete transition 1:		
10. Transition 1: Borg rating of perceived exertion:		
11. Cycle 40 km: Time to complete cycle:		
12. Transition 2: Time to complete transition 2:		
13. Transition 2: Borg rating of perceived exertion:		
14. Transition 2: Blood sample taken		
Time to take blood sample:		
15. Transition 2: Finger prick for capillary lactate, write value:		
16. Run 10 km: Time to complete run:		
17. Finish line: Time to complete entire triathlon:		
18. Finish line: Borg rating of perceived exertion:		
19. Finish line: Blood sample taken		
20. Finish line: Finger prick for capillary lactate 3 minutes:		
21. Finish line: Finger prick for capillary lactate 6 minutes:		
22. Finish line: Finger prick for capillary lactate 9 minutes:		
23. Finish line: Finger prick for capillary lactate 12 minutes:		
24. Finish line: Finger prick for capillary lactate 15 minutes:		
25. Finish line: Profile of Mood State questionnaire completed (POMS)		
26. Finish line: Hand in food record 2		
27. PRIZE GIVING		

APPENDIX 3.4 EVENT PLAN



UNIVERSITEIT • STELLENBOSCH • UNIVERSITY
jou kennisvennoot • your knowledge partner

To:

Cape Town Events Office (CTF&EO)
8th Floor, Waldorf Building
St Georges Mall
Cape Town
PO Box 16548
Vlaeberg
8018
South Africa
Tel: +27 21 483 9013
Date: 15 March 2011

*Application for event
Research Triathlons: 8 & 22 May 2011*

Contact:

Sunita Potgieter

Tel: 021 938 9474 or 082 335 3650

E-mail: sunita@sun.ac.za



Fakulteit Gesondheidswetenskappe • Faculty of Health Sciences



Verbind tot Optimale Gesondheid • Committed to Optimal Health

Department of Human Nutrition • Departement Menslike Voeding

Posbus/PO Box 19063 • Tygerberg 7505 • Suid-Afrika/South Africa

Tel.: +27 21 938 9259 • Faks/Fax: +27 21 9332991

Webblad / Web page: www.sun.ac.za/nutrition; www.sun.ac.za/fricus

Event Plan:

1. Description of the event

Type of events:

We would like to host two triathlon events at the same venue in Gordon's Bay, 2 weeks apart. The triathlons are organized for the purpose of doing research. We would like to determine how caffeine supplementation influences Olympic distance triathletes and their triathlon performance.

The triathlons are organized in conjunction with **Western Province Triathlon Association (WPTA)** and will be sanctioned by **Triathlon South Africa (TSA)**. **Referees for the event will be provided by WPTA.**

Contact at WPTA:

Tony Bradford, 084 583 8989 or bradfordanthony@absamail.co.za

The study is a randomized controlled double blind clinical trial with a cross over design, and the main aim is to determine to what extent and through which mechanism(s) caffeine supplementation influences Olympic distance triathlon performance in male and female triathletes living in the Western Cape. Data will be collected during the two triathlons which will be held 14 days apart at the same venue in Gordon's Bay, Western Cape Province, South Africa. Both triathlons will consist of a sequential 1.5 km swim, 40 km cycle and 10 km run. The triathlon(s) are organized for the sole purpose of doing this research project and will take place on 8 and 22 May 2011

Important!

Please also refer to the event plan (map) submitted with this event plan.

Dates: 8 and 22 May 2011

Duration: The athletes will arrive at 07h00 and we will be finished by 12h30.

Locality: Gordon's Bay, Helderberg, Western Cape

Venue:

We would like to host both triathlons in Gordon's Bay. The triathlon will consist of a 1.5 km swim in the Gordon's Bay sea (start will be in front of the Spur in Gordon's Bay), followed by a 40 km cycle (along Gordon's Bay road toward Strand) and a 10 km run (along the beach toward Bikini Beach).

Between each leg of the triathlon, the athletes will come back to the transition zone, which will be set up in the parking area in front of the sea in Gordon's Bay. The transition zone will be restricted to athletes and referees of the race. Pathcare will have a mobile clinic set up in the transition area, which will be restricted to the participants and the nurses. The mobile clinic is there to draw blood samples from the athletes at various time points during the triathlon. Pathcare will handle the sharp objects as per their protocol.

The **anticipated number of spectators** is 100-150 people. This will only include the families / support teams of the athletes participating in the triathlons.

We will only be allowing **80 participants** to take part in the event. As these events are research triathlons.

2. Event program (full details and times,)

Person responsible for all aspects of both the events:

Sunita Potgieter

Office phone: 021 938 9474

Cell phone: 082 335 3650

E-mail: sunita@sun.ac.za

Event program with full details and times:

Time:	Triathlon 1: 8 May 2011	Triathlon 2: 22 May 2011
07h00	Athletes and participants arrive at the event	Athletes and participants arrive at the event
07h00 – 07h45	Baseline blood sample at the Pathcare mobile clinic Bike racking	Baseline blood sample at the Pathcare mobile clinic Bike racking
07h45	Caffeine / placebo supplementation	Caffeine / placebo supplementation
08h30	Start of race – 1.5 km swim	Start of race – 1.5 km swim
08h45 – 09h15	Anticipated finish of the swim Athletes move through transition 1 Start of the 40 km cycle	Anticipated finish of the swim Athletes move through transition 1 Start of the 40 km cycle
09h40 – 10h45	Anticipated finish time of the 40 km cycle Athletes move through transition 2 Athletes move through the Pathcare mobile clinic Start of the 10 km run	Anticipated finish time of the 40 km cycle Athletes move through transition 2 Athletes move through the Pathcare mobile clinic Start of the 10 km run
10h10 – 12h00	Anticipated finish of the 10 km run Athletes move through the Pathcare mobile clinic Finish of the race	Anticipated finish of the 10 km run Athletes move through the Pathcare mobile clinic Finish of the race
12h30		Prize giving

3. Layout of the event**Stages:**

No stages will be built for the purpose of these events.

Marquees:

Pathcare will have a mobile clinic that they will set up.

We will have a registration site under a gazebo where athletes need to sign indemnity and consent forms, register for the race and body marking can take place.

Fencing:

There will be fencing around the transition area.

Crowd barriers:

The transition zone will be clearly marked (barracked) and only athletes and referees will be permitted in the transition zone.

We do not anticipate a big crowd.

Vendors:

No vendors will be permitted at the event

Catering:

Food parcels and drinks will be given to the marshals, referees and volunteers.
Drinks (water & sports drinks) will be supplied to the athletes during and after both triathlons.
No other catering or vendors will be permitted at the event.

Lockdown:

The athletes need to register between 07h00-07h45.

The transition area will be open for bike racking from 07h00-07h45. After that the transition area will be closed. Only athletes will be allowed in the transition area during designated times. The transition area will open for people to remove their bikes as soon as the last triathlete has finished the cycle leg of the triathlon.

VOC location:

Administration place indicated on event map. This is where the traffic, medical and race organizers will be stationed.

Ticket selling booths:

There will be no ticket selling booths.

4. Transport management plan

The race organizer met with the Gordon's Bay Traffic Department (Mr. Esterhuizen 021 856 8002) in December 2010 to discuss the possibility of hosting a triathlon in Gordon's Bay. They agreed and said they would be willing to help out, once approval has been obtained from the City Council.

Road closures (and times):

To be finalized by the Gordon's Bay Traffic Department.

Parking areas:

The parking area in front of the beach at Gordon's Bay will be used for the transition area and the Pathcare mobile clinic.
Additional parking is available on the opposite side of the Spur, as well as in the street. Additional parking is indicated on the event map.

Route plan:

Swim: 1.5 km

The swim will be one out –and back loop in the Gordon's Bay Sea. The National Sea Rescue Institute (NSRI) will be responsible for measuring the distance of the swim

the morning of both triathlons and they will provide lifeguards for water safety during the entire swim leg of both triathlons.

(Contact person: Nigel: 083 625 0481 / matilandvinegar@intekom.co.za)

The swim will start after high tide*

*Table indicating high tides

	8 May 2011	22 May 2011
Cape Town	06h07	06h33
Simons Town	06h04	06h29
Hermanus	06h07	06h32

The start of the swim will be at **08h30**

The swim will last between 15 – 60 minutes. Therefore the anticipated time that the athletes will have completed the 1.5 km swim, move through transition 1 and start the 40 km cycle route will be **08h45 – 09h15**.

Swim route plan: The swim will be in a triangle route as shown on the event map.

Cycle: 40 km

The cycle will consist of 4 laps of 10 km to make up the 40 km cycle route. The cycle route will start at the parking lot in front of the beach in Gordon's Bay. The athletes will cycle along Beach Road toward Faure Marine Drive (R44) in the direction of Strand. They will continue along Gordon's bay road for 5 km after which they will turn and head back toward Gordon's Bay beach. They will turn in front of the BP garage in Beach Road. They will complete this lap 4 times. On the last lap, they will continue past the BP garage and go back to the transition zone, which is in Beach Road. Additional signage on the cycle route will be provided by WPTA to mark the cycle route.

Intersections where traffic officers are required:

A total of **8 Traffic officers** are needed at the following sections on the cycle route:

- Cycle: Beach Road and Sir Lowry Road (1 officers needed)
- Cycle: Beach Road and Faure Marine drive (1 officer needed)
- Cycle: Faure Marine and Lemoenboom street (traffic circle) (2 officers needed)
- Cycle: Faure Marine and Broadlands road (1 officer needed)
- Cycle: Faure Marine and Beach Road (Strand) (1 officer needed)
- Cycle: Faure Marine and de Kock Hammond Street (2 officers needed)

Marshals / Volunteers will be placed at all the other intersections.

Cycling 5 km out (toward Strand), total 4 X 10 km loops

- Beach Road and Sir Lowry Road
- Beach Road and Faure Marine Drive
- Faure Marine Drive and Cassia Road
- Faure Marine Drive and Bloubos Road
- Faure Marine Drive and Acacia Road
- Faure Marine Drive and Octopus Road
- Faure Marine Drive and Jan Bruin Road
- Faure Marine Drive and Flow Street
- Faure Marine Drive and Dolphin Street
- Faure Marine Drive and Crab Street
- Faure Marine Drive and Barracuda Street
- Faure Marine Drive and Broadlands Street

Turn at Hammond Street

- Right turn at Hammond Street
- Right turn at Van Rhee de Street
- Right turn at Nolte Street
- Left turn into Faure Marine drive

Cycle 5 km along Faure Marine Drive toward Gordon's Bay Beach

- Faure Marine Drive and Oosterling Street
- Faure Marine Drive and Brewery Street
- Faure Marine Drive and Bosch Street
- Faure Marine Drive and Naomi Street
- Faure Marine Drive and Abbotoir Street
- Faure Marine Drive and Webb Street
- Faure Marine Drive and Onverwacht Street
- Faure Marine Drive and Rusthof Road
- Faure Marine Drive and Broadlands
- Faure Marine Drive and Albatros Road
- Faure Marine Drive and Barracuda Street
- Faure Marine Drive and Ebb Street
- Faure Marine Drive and Jan Bruin Road
- Faure Marine Drive and Octopus Road
- Faure Marine Drive and Bloubos Road
- Faure Marine Drive and Disa Road
- Faure Marine Drive and Fuchsia Road
- Faure Marine Drive and Hibiscus Road
- Faure Marine Drive and Lemoenboom Road (traffic circle)
- Faure Marine Drive and Robina Road

- Faure Marine Drive and Ulex Road
- Faure Marine Drive and Beach Road
- Beach Road and Sir Lowry Road

The start of the cycle will be at **08h45** and last until **10h45**. This is the anticipated finish time of the slowest cyclist.

Cycle route plan: The cycle route will be 4 X 10 km laps as indicated on the event map.

Run: 10 km

The 10 km run will start at the transition zone in front of the Gordon's Bay Beach. The athletes will run along Beach Road heading toward Bikini beach. The 10 km run will consist of 3 X 3.3 km laps.

The start of the 10 km run will be at around **09h40** and last until **12h00**.

Run route plan: The run route will be 3 X 3.3 km laps as indicated on the event map.

Intersections where traffic officers are required:

A total of 2 **Traffic officers** are needed at the following sections on the run route:

- Run: Beach Road and Sir Lowry Road (1 officer needed)
- Run: Bikini beach road (1 officer needed)

Additional intersections will be marshaled (no traffic officers needed):

- Beach Road and Sir Lowry Road
- Beach Road and Van Der Byl Road
- Beach Road and Cilliers Street
- Beach Road and Jannie Storm Street
- Beach Road and Hahn Road
- Beach Road and Bikini Beach Road
- Turn around at Bikini Beach
- Beach Road and Sir Lowry Road

Emergency access routes:

Indicated on the event map.

Fire Brigade and Ambulance will have access to the event via the N2, Sir Lowry's Pass Road, Faure Marine Drive, Beach Road and Van der Byl Road.

Emergency vehicle parking areas:

Indicated on the event map (Gordon's Bay Security response vehicle, Ambulance services)

5. Emergency

Medical plan:

Gordon's Bay Security Trust Ambulance service will provide the following at both events:

- 2 Medics
- 1 Ambulance on standby
- 1 Primary medical response vehicle

Contact details:

Gordon's Bay Security Trust Ambulance Service
2 Link Road
Mansfield Industrial
Gordon's Bay
7151

Telephone: 021 856 0214

Fax: 021 856 0213

Contact person: Charl Cilliers (083 4517629)

Security plan:

VETUS SCHOLA PROTECTION SERVICES (PTY) LTD have been contracted to oversee security at the events. They will offer support in the fields of visible presence, risk and disaster management and integrated security solutions.

Evacuation plan:

Emergency evacuation areas indicated on the event map.

Facility emergency plan:

Not applicable

Civil aviation application:

Not applicable

6. Vendors / caterers

No vendors or caterers will be permitted on site.

Food parcels and drinks for volunteers, marshals and referees will be pre-packed.

Water and sports drinks will be supplied to the athletes.

7. Health requirements

Vendor license:

Not applicable

Food integrity:

Not applicable

Certificate of acceptability:

Not applicable

Tobacco control:

The events are held outside. No tobacco control needed.

Ablution facilities and /or mobile toilets:

There are public toilets available at the beach where the triathlons will be held (Gordon's Bay).

8. Completed application forms for

Liquor license:

Not applicable

Noise exemption:

There will be little or no noise during the two events. The race officials will use a megaphone to communicate with the athletes. There will be no music.

Erection of stages / marquees:

No stages or marquees will be erected. We will only have two gazebos – one for Pathcare mobile clinic and one for registration purposes.

9. Service requirements

Electricity:

There will be electricity available on site. Once approval of the City council has been obtained, the organizer plans to have a meeting with the owner of the Spur in Gordon's Bay to arrange for electricity needed being arranged through them. Electricity will only be needed for the Pathcare mobile clinic.

Water:

Water will be provided to the athletes during and after the triathlons. This will be supplied by a water company.

There is running water and taps on site.

Waste management plan:

The organizers and volunteers will make sure the area where the triathlons are held is clean with no waste.

We will place dustbins everywhere and have a dedicated person ensuring that everything is clean. Additional boxes with black bags will be provided to ensure adequate waste management.

The black bags and waste will be taken back to Stellenbosch University for disposal.

Prior arrangements for cleaning venue:

Additional volunteers have been allocated to clean the venue after both triathlons.

Bins

There are bins available on the beach.

Receipt slip from landfill site:

This will be handed in after both events

10. Event communication plan

Ticket selling strategy:

Not applicable

Medical plan:

Gordon's Bay Security Trust Ambulance service will provide the following at both events:

- 2 Medics
- 1 Ambulance on standby
- 1 Primary medical response vehicle

Contact details:

Gordon's Bay Security Trust Ambulance Service
2 Link Road
Mansfield Industrial
Gordon's Bay
7151

Telephone: 021 856 0214

Fax: 021 856 0213

Contact person: Charl Cilliers (083 4517629)

Accreditation:

The event will be sanctioned by Triathlon South Africa (TSA) and Western Province Triathlon Association (WPTA). WPTA referees will oversee both events. Different official's responsibilities will be identified by wearing name tags and t-shirts.

Any specific requirements:

None.

11. Environmental protection plan

The events will have no negative effect on the environment.

There will be no damage to the coral.
A waste management plan is in place to protect the environment.
There will be not cutting of vegetation.

12. Community participation plan

Once approval has been granted from the City Council, the organizer will contact all businesses and residences in the area and let them know about the events.

13. Indemnity forms

Indemnity form is completed and attached.
The athletes will all sign indemnity and an informed written consent form before every triathlon.

Public Liability insurance confirmation letter

I await your response at your earliest convenience.

Yours faithfully,




Mrs. Sunita Potgieter

Signed at Tygerberg on 18 April 2011



APPENDIX 3.5 EVENT PERMIT



CITY OF CAPE TOWN | SOUTHERN SCAVENGERS | CSD: 00000000

<p>2nd Floor, Village House 20 St George's Mall Cape Town 7800 PO Box 18540, Vredeburg, 2012</p> <p>Sub: Mr. Julian Fredericks Tel: 021 421 2372 Fax: 021 518 2403</p> <p>Ref: EO#21 V11.F</p>	<p>2nd Floor, Village House 20 St George's Mall Cape Town 7800 PO Box 18540, Vredeburg, 2012</p> <p>Sub: Mr. Julian Fredericks Tel: 021 421 2372 Fax: 021 518 2403</p>	<p>2nd Floor, Village House 20 St George's Mall Cape Town 7800 PO Box 18540, Vredeburg, 2012</p> <p>Sub: Mr. Julian Fredericks Tel: 021 421 2372 Fax: 021 518 2403</p>
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Filename: Research Triathlons - Gordons Bay - v 22 May 2011 (Amended)

ECONOMIC & HUMAN DEVELOPMENT – FILM AND EVENTS PERMIT OFFICE

Date: 2011-05-06

PER EMAIL: sunits@sun.ac.za

The Event Organizer
"RESEARCH TRIATHLONS"
c/o Department of Human Nutrition
Faculty of Health Sciences
Stellenbosch University
P.O. Box 19063
Tygerberg
7505

ATT: Ms Sunita Potgieter (CELL: 082 335 3650)

Dear Madam

Your application received on 16 March 2011 refers:

PERMISSION IS HEREBY GRANTED TO THE DEPARTMENT OF HUMAN NUTRITION – STELLENBOSCH UNIVERSITY TO HOST AN EVENT: "RESEARCH TRIATHLONS" WITH THE FOLLOWING DETAILS:

Date: 22 May & 5 June 2011

Time: 07h00 – 14h00

Location: Gordons Bay

Route: As per Annexure A

AMENDED

I have pleasure in advising that, in so far as approvals by the City of Cape Town are required, there is no objection to your organisation hosting the abovementioned event.

The Municipality of the City of Cape Town hereby grants permission on the following conditions:

SPECIAL CONDITIONS:

- I. Immediate Emergency Vehicular access and egress to be guaranteed at all times, i.e. during the event as well as during setup and strike down;
- II. All requirements as per the Community Fire Safety By-law including Community Fire Safety Amendment By-law are to be complied with;
- III. The conditions stipulated by the Fire Safety Branch of the Cape Town Fire And Emergency Service Department must be complied with at all times;
- IV. Marshalls are to be equipped with reflective vests and red flags and to fall under the direction of Traffic/Law Enforcement personnel.
- V. Marshalls are not to stop/regulate traffic, they are rather to stop/manage participants;
- VI. Notwithstanding the deployment of the Traffic Officers, the Event Organizer will ensure

sufficient marshals are deployed along the route to ensure the safety of participants and spectators.

- VII. All traffic laws to be obeyed.
- VIII. Pedestrian and vehicular traffic must not be unduly disrupted.
- IX. The Event Organizer will ensure that the activation/deployment of appropriate NSRRS/First Aid/Life saving personnel along the sea leg/s of the event to ensure the safety of participants and/or spectators.
- X. All conditions attached to the permission for the use of the venue issued by the City's Sport, Recreation and Amenities Branch must to be complied with at all times.

Standard Conditions:

1. that this approval will require the deployment of traffic officers to facilitate traffic control during the event. In regard thereto kindly contact the following Traffic Official immediately to arrange for a written quotation to be issued:

Inspector G. Morris - 021 356 3005
2. that the Municipality shall be indemnified against all actions, suits, proceedings, claims, demands, costs and expenses arising out of the permission given;
3. that precautions shall be taken to avoid damaging any of the Municipality's roads/street surfaces, kerbs, pavements, etc;
4. that the temporary closure of the Municipality's roads/streets must be facilitated with barricades, warning signs and lights in accordance with the requirements as contained in Chapter 13 of the SA Road Traffic Sign Manual (SARTSM) for the duration of the event and be also placed with the approval of the Traffic Manager in terms of those;
5. that preparation of any refreshments shall take place on private property and not on any public street or road;
6. that applicants shall, at the conclusion of the event, clean the area to the satisfaction of the Municipality;
7. that any additional permissions that may be required in terms of those laws governing the assembly of persons in public places must also be applied for;
8. that applicants must ensure that the relevant laws relating to Noise, use of Loudspeakers, Traffic etc. are complied with at all times;
9. that applicants must comply with the requirements and instructions received from any of the Municipality's uniformed law enforcement agencies or its own law enforcement officers at all times;
10. that volume of any amplified sounds provided shall be so modulated so as not to cause a nuisance to persons in the surrounding area and must be terminated at the latest as indicated herein on each event of the day;
11. that applicants must see to the removal of litter and obstructions at the conclusion of the event. The cleaning of the road and collection of waste will be for your account. Also kindly arrange for the provision of adequate toilet facilities;
12. that applicants must ensure that access to / egress from the road / street for emergency vehicles shall be maintained at all times. A 4 metre wide Emergency Vehicular Lane dedicated to Emergency Vehicles Access only, is to be provided and kept unobstructed. THE FIRE AND EMERGENCY CONTROL NUMBER are:
 - 107 - Telkom line users
 - 021 480 7700 - Cellphone users

All Fire Hydrants, Water Pipes, Hydrant Marking Plates or Decals are to remain accessible and unobstructed at all times.

13. that the right of passage along the road cannot be denied to any person wishing to exercise that right;
14. that it is strictly prohibited to drive any pegs, stakes or spikes into the road / street or roadway surfaces for whatever reasons there might be;
15. that it is strictly prohibited to mark with paint or other materials the road / street or roadway surfaces for whatever reasons there might be;
16. that this permit shall be revoked in the event of any breach of the above conditions;
17. that in the event of there being any damage to Municipal property, the applicant shall be held liable for the costs incurred to reinstate the area used to its condition prior to the damage. The costs of any Municipal services rendered in respect of such damage are payable directly to the appropriate Department / Branch that rendered the service;
18. that applicants who are liable to pay for costs arising from the assistance rendered by any of the Municipal services for which a charge may be levied must arrange the payment thereof to the appropriate Department / Branch that rendered the service;
19. No advertising sign may be displayed without a written approval from the Environmental and Heritage Management Branch in terms of the Outdoor Advertising and Signage By-Law of 2001; and any such sign displayed without written approval, may be removed by the City and the Event Organizer or Advertiser will be billed with the applicable removal charge and/or may be fined in addition, in accordance with the By-Law Offences and Penalties Clause.

NB:

The City reserves the right to, at its sole discretion, add to, alter or withdraw any of the conditions attached to this permit or to withdraw the permit in its entirety should it deem the conditions attached hereto have not been satisfactorily complied with, or that such action to be in the interests of public safety.

Yours faithfully


For CITY MANAGER

Copy to:

The Manager: Traffic (East)	The Manager: Cleansing
The Manager: Fire and Rescue	The Manager: Environmental Health
The Manager: Disaster Risk Management	The Manager: Metro Police
SAPS: WIP Ops	

APPENDIX 3.6 CAFFEINE CERTIFICATE OF PURITY



Certificate of Analysis

Caffeine 70%

Date of Manufacture: 02/15/11*

Lot No. CAF 150-70-49

Criteria	Specification	Result
Appearance	Off White	Passes
Assay	70% \pm 2%	70.3%
Encapsulant, Vegetable Oil	30% \pm 2%	29.7%
Encapsulant Melt Point	150°F-157°F (66-69° C)	152.3° F (66.8° C)
Particle Size	2% max on 20 mesh	Passes

*Expiration date: 24 months from date of manufacture

Approved Kurt Co

3621 Aerial Way Drive, Roanoke, VA 24018
p 540.904.6635 f 540.904.6673 e inquire@maxxperform.com
www.maxxperform.com

APPENDIX 3.7 BORG SCALE RATING OF PERCEIVED EXERTION (RPE)

Borg Scale Rating of perceived exertion		
Subject Reference number: _____		
	LEVEL	DESCRIPTION
	20	Maximum
	19	Very, very hard
	18	
	17	Very hard
	16	
	15	Hard
	14	
	13	Somewhat hard
	12	
	11	Fairly light
	10	
	9	Very light
	8	
	7	Very, very light
6		

APPENDIX 3.8 PROFILE OF MOOD STATES (POMS) QUESTIONNAIRE

Profile of mood state (POMS) questionnaire

Subject Reference number: _____

Name: _____ Date: _____

PROFILE OF MOOD STATE (POMS)
Developed by Douglas M. McNair, Maurice Lorr & Leo F. Droppleman

Please circle the one number for each word which best describes HOW YOU HAVE BEEN FEELING DURING THE PAST WEEK INCLUDING TODAY

		Not at all	A little	Moderately	Quite a bit	Extremely
1	Tense	0	1	2	3	4
2	Worn out	0	1	2	3	4
3	Lively	0	1	2	3	4
4	Shaky	0	1	2	3	4
5	Listless	0	1	2	3	4
6	Active	0	1	2	3	4
7	On Edge	0	1	2	3	4
8	Energetic	0	1	2	3	4
9	Panicky	0	1	2	3	4
10	Relaxed	0	1	2	3	4
11	Uneasy	0	1	2	3	4
12	Restless	0	1	2	3	4
13	Fatigued	0	1	2	3	4
14	Nervous	0	1	2	3	4
15	Cheerful	0	1	2	3	4
16	Exhausted	0	1	2	3	4
17	Anxious	0	1	2	3	4
18	Sluggish	0	1	2	3	4
19	Weary	0	1	2	3	4
20	Alert	0	1	2	3	4
21	Full of pep	0	1	2	3	4
22	Carefree	0	1	2	3	4
23	Vigorous	0	1	2	3	4
24	Bushed	0	1	2	3	4

APPENDIX 3.9 HABITUAL CAFFEINE FOOD FREQUENCY HISTORY

Habitual caffeine intake questionnaire

Subject Reference number: _____

We would like to find out your habitual caffeine intake. This information is important to know, as the efficacy of caffeine supplementation depends on your habituation to caffeine.

Please think carefully about the food and drinks that you have consumed during the past 6 months. The questionnaire goes through a list of foods and drinks and I would like you to please indicate the following:

- If you eat these particular foods or drinks,
- How the food or drink is prepared (by you or whoever prepares the food),
- How much of the food and you eat and drink at a time, and
- How many times a day you eat the food and drink and if you do not eat it every day, how many times a week or a month it is eaten?

To help you to describe the amount of a food, you can report it as household measures (one cup, one teaspoon etc.) Please indicate which measure is the closest to the amount eaten, or if it is smaller, between sizes or bigger than the models.

Amounts must be reported as cups (c), tablespoons (T), serving spoons (SP) or teaspoons (t).

It is important to know that: THERE ARE NO RIGHT OR WRONG ANSWERS **AND** EVERYTHING YOU WRITE DOWN IS CONFIDENTIAL.

Measures

- 1t = 1 rounded teaspoon
- 1T = 1 rounded tablespoon (15ml)
- 1SP = 1 rounded serving spoon (30ml)
- c = measuring cup (250ml)
- s/s = small size
- m/s medium size
- L/s = large size

	FOOD	QUANTITY (g/ml) (office use)	USUAL AMOUNT EATEN (HHM)	Times per day	Days per week	Times per month	Seldom / Never	USUAL AMOUNT EATEN (g) (office use)	Caffeine content mg/ 100g (office use)	Total amount of caffeine (daily) (office use)
Caffeine containing foods	Caramels	6 pieces = 40g							7mg/100g	
	Chocolate bars, Please specify:	1 bar = 50g							66mg/100g	
	Fudge	1 piece = 17g							16mg/100g	
	Chocolate cake:	1 slice = 100g							7mg/100g	
	Chocolate Cup Cakes (chocolate frosting)	1 cupcake = 50g							3mg/100g	
	Brownies	1 square = 56g							2mg/100g	
	Chocolate chip cookies	1 cookie = 12g							11mg/100g	
	Cookies, sandwich type with cr�me filling	1 cookie = 10g							13mg/100g	
	Cookies, chocolate wafers	1 wafer = 6g							7mg/100g	
	Sweetie pie	1 pie = 39g							5mg/100g	
	Chocolate coated doughnut	1 doughnut = 60g							2mg/100g	
	Chocolate eclairs	1 �clair = 100g							2mg/100g	
	Coco Pops	1 bowl = 30g							2mg/100g	
	Chocolate Pronutro	Serving = 37g							23mg/100g	
	Chocolate ice-cream	Serving = 58g							3mg/100g	
	Chocolate spread	2 tbsp = 37g							7mg/100g	

	FOOD	QUANTITY (g/ml) (office use)	USUAL AMOUNT EATEN (HHM)	Times per day	Days per week	Times per month	Seldom / Never	USUAL AMOUNT EATEN (g) (office use)	Caffeine content mg/ 100g (office use)	Total amount of caffeine (daily) (office use)
Caffeine containing drinks	Energy gels (e.g. Gu)									
	Chocolate syrup	2tbsp = 39g							14mg/100g	
	Chocolate frozen yoghurt	Serving = 174g (1 cup)							3mg/100g	
	Instant coffee	1 rounded tsp = 1.8g							3142mg/100g	
	Filter coffee	1 cup = 237g							40mg/100g	
	Espresso	Serving = 30g							212/100mg	
	Cappuccino powder	2.5tsp = 27g							302mg/100g	
	Café Mocha (coffee & cocoa)	1 cup = 237g							218mg/100g	
	Decaffeinated filter coffee	1 cup = 237g							1mg/100g	
	Decaffeinated instant coffee	1 cup = 237g							122mg/100g	
	Other coffee, Please specify:									
	Cacao drinks Please specify type:	Serving = 15g							19mg/100g	
	Milo	3 heaped tsp = 21g							37mg/100g	
	Hot chocolate Please specify type:	3 heaped tsp = 28g							18mg/100g	
	Ceylon tea (instant)	1 serving = 14.4g							1344mg/100g	

	FOOD	QUANTITY (g/ml) (office use)	USUAL AMOUNT EATEN (HHM)	Times per day	Days per week	Times per month	Seldom / Never	USUAL AMOUNT EATEN (g) (office use)	Caffeine content mg/ 100g (office use)	Total amount of caffeine (daily) (office use)
	Black tea	Serving = 29.6g							20mg/100g	
	Iced Tea Please specify flavour:	500ml = 368g							5mg/100g	
	Energy drinks (e.g. Red Bull) Please specify type:	1 can = 250g							30mg/100g	
	Soft drinks Please specify type:	1 can = 369g							8-15mg /100g	
	Chocolate milk (e.g. Steri stumpi)	Serving = 266g							3mg/100g	
	Coffee liqueur	1 shot = 34.8g							26mg/100g	
	Nesquick powder	Serving = 22g							8mg/100g	
	Chocolate milkshake	Serving = 376g							1mg/100g	

APPENDIX 3.10 MENSTRUAL HISTORY QUESTIONNAIRE

Female athletes:	
Menstrual History	
Have you ever had a menstrual period?	<input type="checkbox"/> YES <input type="checkbox"/> NO
How old were you when you had your first menstrual period?	_____ years
If you participate in sports or activities competitively, did you have your first menstrual period before or after you began training for your sport or activity?	<input type="checkbox"/> Before <input type="checkbox"/> After <input type="checkbox"/> Not applicable
In the past, about how many times per year did you get your menstrual period?	<input type="checkbox"/> 10-13 times per year <input type="checkbox"/> 6-9 times per year <input type="checkbox"/> 4-6 times per year <input type="checkbox"/> 1-3 times per year
Have you ever gone for more than 3 months without having a menstrual period?	<input type="checkbox"/> YES <input type="checkbox"/> NO
If you checked YES for the previous question, please answer the following questions:	
Where this due to pregnancy?	<input type="checkbox"/> YES <input type="checkbox"/> NO
If yes, answer the following questions:	
a. How many pregnancies have you had up to date?	
b. Where there any complications with your pregnancy/ies?	<input type="checkbox"/> YES <input type="checkbox"/> NO
c. If, yes describe here:	
d. Do you have a history of severe postpartum bleeding?	<input type="checkbox"/> YES <input type="checkbox"/> NO
If you have gone for more than 3 months without having a menstrual period but it was not due to pregnancy, please answer the following questions:	
a. How old were you when you missed ≥ 3 menstrual periods?	_____ years
b. How many months or years did you go without a menstrual period?	_____ months or years
c. Did you see a doctor during this time period?	<input type="checkbox"/> YES <input type="checkbox"/> NO
d. Did your doctor prescribe some form of contraception to regulate your menstrual periods?	<input type="checkbox"/> YES <input type="checkbox"/> NO <input type="checkbox"/> N/A
e. Was the time before you started to skip your periods a very stressful time or did a stressful event take place?	<input type="checkbox"/> YES <input type="checkbox"/> NO
f. Do you take any of the following medications?	
a. Antipsychotics	<input type="checkbox"/> YES <input type="checkbox"/> NO
b. Tricyclic antidepressants	<input type="checkbox"/> YES <input type="checkbox"/> NO
c. Calcium channel blockers	<input type="checkbox"/> YES <input type="checkbox"/> NO
d. Methyldopa	<input type="checkbox"/> YES <input type="checkbox"/> NO
e. Reserpine	<input type="checkbox"/> YES <input type="checkbox"/> NO
f. Digitalis	<input type="checkbox"/> YES <input type="checkbox"/> NO
g. Chemotherapeutic drugs	<input type="checkbox"/> YES <input type="checkbox"/> NO

How many menstrual periods have you had a. In the past 12 months? _____ b. In the past 6 months? _____	
Current Menstrual Status	
Currently, how would you describe your menstrual cycle? In order to determine the number of days your cycle lasts, begin with the first day of bleeding and count the number of days until the next month when you began bleeding again. <input type="checkbox"/> I am very regular (every 26-35 days) <input type="checkbox"/> I am somewhat regular (every 21-25 days) <input type="checkbox"/> I am very irregular (every 36-45 days) <input type="checkbox"/> I do not have a menstrual cycle (no cycle for longer than 3 months)	
When was your last cycle? _____	
If you do not have a menstrual cycle, choose all the possible reasons that apply: <input type="checkbox"/> Training intensity <input type="checkbox"/> Contraceptive use <input type="checkbox"/> Reproductive disorder <input type="checkbox"/> I don't know <input type="checkbox"/> Other, please specify _____	
How would you describe the length of your menstrual cycle during a usual month (check one) <input type="checkbox"/> the same as always <input type="checkbox"/> shorter than usual <input type="checkbox"/> longer than usual	
How would you describe your menstrual bleeding during a usual month's cycle (check one) <input type="checkbox"/> the same as always <input type="checkbox"/> lighter than usual <input type="checkbox"/> heavier than usual <input type="checkbox"/> No cycle for > 3 months	
Does your menstrual cycle change with your training? <input type="checkbox"/> YES <input type="checkbox"/> NO <input type="checkbox"/> Not applicable	
If you checked YES , choose all that apply: <input type="checkbox"/> Longer cycle (>35 days) <input type="checkbox"/> Skipping a cycle <input type="checkbox"/> Shorter cycle (<21 days) <input type="checkbox"/> Heavier bleeding <input type="checkbox"/> Absence of 3 or more consecutive cycles	
Does your menstrual cycle change during your competition season?	<input type="checkbox"/> YES <input type="checkbox"/> NO <input type="checkbox"/> Not applicable
If you checked YES , choose one of the following: <input type="checkbox"/> Longer cycle (>35 days) <input type="checkbox"/> Skipping a cycle <input type="checkbox"/> Shorter cycle (<21 days) <input type="checkbox"/> Heavier bleeding <input type="checkbox"/> Absence of 3 or more consecutive cycles <input type="checkbox"/> Other, please specify: _____	

<p>Do you currently use contraception/birth control (e.g., oral contraceptive pills, rings, implants, injections)?</p> <p><input type="checkbox"/> YES, nr. of yrs____ <input type="checkbox"/> NO <input type="checkbox"/> Not applicable</p>	
<p>If YES, what are you using them for?</p> <p><input type="checkbox"/> Birth control <input type="checkbox"/> Regulate cycle <input type="checkbox"/> Both <input type="checkbox"/> other</p>	
<p>If NO, have you used contraception/birth control (e.g., oral contraceptive pills, rings, implants, injections) in the past?</p> <p><input type="checkbox"/> YES, nr. of yrs____ <input type="checkbox"/> NO- never used <input type="checkbox"/> N/A</p>	
<p>If YES, what did you use them for in the past?</p> <p><input type="checkbox"/> Birth control <input type="checkbox"/> Regulate cycle <input type="checkbox"/> Both <input type="checkbox"/> other</p>	
Have you ever been to a gynaecologist?	<input type="checkbox"/> YES <input type="checkbox"/> NO
<p>If you checked YES for the previous question, was any reproductive disorder identified/diagnosed?</p> <p><input type="checkbox"/> YES <input type="checkbox"/> NO If YES, please give more details:_____</p>	
<p>Do you currently monitor your menstrual cycle?</p> <p><input type="checkbox"/> YES <input type="checkbox"/> NO</p>	
<p>If you checked YES for the previous question, how many months or years have you monitored your menstrual cycle?</p> <p>Nr. _____ years OR Nr. _____ months</p>	

APPENDIX 3.11 DEMOGRAPHIC QUESTIONNAIRE

Demographic questionnaire
Subject Reference number: _____

Please complete the following demographic information. This information will be kept strictly confidential. Your contact details are important for the researcher to contact you during the time of the data collection. The medical and in case of emergency details is important in the event of a medical emergency during either triathlon 1 or triathlon 2. All information will be destroyed as soon as the data collection has been completed.

Personal details:	
Age:	
Gender:	
Ethnicity:	
Birth date:	
Address:	
E-mail address:	
Telephone number:	
Cell phone number:	
Athlete registration details:	
Provincial registration:	
Provincial registration number:	
Elite ranking:	
Medical aid details:	
Medical aid:	
Medical aid number:	
Contact in case of an emergency:	
Name:	
Relationship:	
Contact details:	

APPENDIX 3.12 TRAINING REGIME QUESTIONNAIRE

Training regime questionnaire	
Subject Reference number: _____	

1. Do you know your VO_{2max} ? If yes, please give the value:

2. Please indicate how many times per week do you exercise:

3. Please indicate how many training sessions do you have per day:

4. How many hours a week do you spend:

	Hours per week:
Swimming:	
Cycling:	
Running:	
Gym training (weights / resistance training):	
Other (e.g. mountain biking, pilates, yoga, rowing etc.):	
Please specify other:	

5. How many kilometres a week do you:

	Kilometres per week:
Swimming:	
Cycling:	
Running:	
Other (e.g. rowing, kayaking, etc.):	
Please specify other:	

6. Please give a short description of your triathlon career in the past year:

7. What triathlon distance do you prefer racing? Mark with a X:

Super sprint distance:	400m swim, 10km bike, 2.5km run	
Sprint distance:	600/750m swim, 20km bike, 5km run	
ITU Olympic / Standard distance:	1.5km swim, 40km bike, 10km run	
Ironman 70.3 distance (half ironman)	1.9km swim, 90km bike, 21.1km run	
Ironman distance	3.8km swim, 180km bike, 42.2km run	
ITU long course		

8. What are your current goals for the triathlon season ahead?

[illegible]

9. What is your personal best time over an Olympic distance triathlon?

10. What is your personal best ranking in your triathlon career?

11. What is your current ranking?

12. Please list the amount of triathlons you have completed in the past year and the placing you received. If you can, please indicate completion times as well:

[illegible]

APPENDIX 3.13 MEDICAL HISTORY QUESTIONNAIRE

Medical History Questionnaire	
Subject Reference number: _____	
Question:	Yes / No
Have you had a medical illness or injury since your last check up or sports physical?	
Please specify: _____	
Do you have an ongoing or chronic illness?	
Please specify: _____	
Do you have any allergies (food or medication)?	
Please specify: _____	
Do you take over the counter medications?	
Please specify: _____	
Do you take prescribed medication on a permanent or semi-permanent basis (steroids, birth control pills, anti-inflammatory, and antibiotics)?	
Please specify: _____	
Do you use an inhaler?	
Please specify: _____	
Do you use over the counter dietary supplements (vitamins, minerals, herbs, protein)?	
Please specify: _____	
Have you ever been told to give up sports because of a health problem?	
Please specify: _____	
Do you have any of the following:	
High blood pressure	
High cholesterol	
Heart disease	
Diabetes	
Which one of the following dietary supplements have you taken during the past year?	
Multi-vitamin / minerals	
Individual vitamins (e.g. Vitamin C, etc.)	
Individual mineral (e.g. iron, calcium, etc.)	
Protein powders or pills	
Herbals (e.g. Ginseng, Echinacea, etc.)	
Protein drinks or bars	
Energy drinks or bars	
Creatine	
Amino acid pills or powders	
Others, please list: _____	
If you took any dietary supplements during the past year, how frequently did you take them?	

Daily	
Once a week	
Only at specific times (travel, training, etc.)	
Occasionally	
Several times a week	
Other, please specify:	
Check the reasons for using dietary supplements during the past year:	
To make up for an inadequate diet	
To treat a medical condition or injury	
To increase muscle mass / gain weight	
To prevent illness and disease	
To lose weight	
To have more energy	
To enhance exercise performance	
No specific reason	
Other reason, please specify:	

APPENDIX 3.14 THREE DAY FOOD RECORD

<p>Food Record 1</p> <p>Friday 6 May 2011 – Sunday 8 May 2011</p>							
<p>Subject Reference number:</p> <p>_____</p>							
<p>Please read instructions carefully before you start recording</p> <p>Days on which the records should be kept:</p> <table border="1" style="width: 100%; border-collapse: collapse;"> <tr> <td style="width: 35%;">Friday:</td> <td>3 May 2011</td> </tr> <tr> <td>Saturday:</td> <td>4 May 2011</td> </tr> <tr> <td>Sunday:</td> <td>5 May 2011</td> </tr> </table>		Friday:	3 May 2011	Saturday:	4 May 2011	Sunday:	5 May 2011
Friday:	3 May 2011						
Saturday:	4 May 2011						
Sunday:	5 May 2011						
<p><u>Contact information:</u></p> <p>Sunita Potgieter: sunita@sun.ac.za or +27 82 335 3650 or +27 21 938 9474</p>							
<p>I hereby declare that the information completed in this record is truthful.</p> <p>Reporter's signature:</p> <p>_____</p> <p>Print name: Date:</p>							

Instructions:

1. Please return the completed weighed 3 Day Food Record and supplement table to the researcher at the triathlon. You will be asked at the finish line to enter the last details of your race nutrition and then hand in the food record.
2. Remember not to deviate from your normal dietary intake; it may influence the results of the study.
3. Keep your dietary record book with you on the specific days and immediately record food and liquid as it is consumed.
4. When recording, use one line per food item. Begin each day on a new page.
5. Detailed information is essential. Food and liquid consumed must be described in detail and it is important to use the scale given to you by the researcher to accurately record the portion sizes of the food you eat.
6. Where applicable preparation methods or brand names must be given.
7. When eating out or ordering a take-away, the restaurant name and name of dish/item and portion size should be given.
8. All snacks should be noted.

Guidelines:

1. Describe the food e.g. brown rice or white rice.
2. Note whether the bread is home sliced, whether it is thinly, and medium or thickly sliced or machine sliced.
3. Name the bread type, i.e. white, brown, whole-wheat seed loaf and note the dimensions of the rolls.
4. Please indicate the amounts eaten in grams as you weigh it on the scale. Where applicable, household measures can be used, e.g. a teaspoon, dessert spoon, table spoon, ladle, $\frac{1}{2}$ cup, cup.
5. Remember to add whether the spoon was heaped or level.
6. Give the container or wrapper size where possible e.g. a can of cold drink (340ml) OR a small tub of yoghurt (100ml), medium tub yoghurt (175ml) or large tub of yoghurt (500ml). You can read this from the food label.
7. Specify whether the margarine used is either hard (brick), or soft (tub), as well as type e.g. Flora extra light
8. When eating a combined dish e.g. a stew or pasta dish, name all the ingredients of the dish i.e. cottage pie: mashed potato, mince, oil, salt etc. NOTE: quantities of each must be specified!
9. Give preparation method, e.g. one extra large egg fried in tablespoon of oil.
10. Milk/powder milk must be specified as full cream, 2% or skimmed milk. Give the brand name in case of milk powder.
11. Remember to give names and amounts of all cold drinks, juice and alcoholic beverages

See example below:

Day: Wednesday 6 April 2011			
Time:	Item eaten:	Amount	Official Use:
8 am	Mealie porridge, soft	½ cup or 125g	
	2 % milk	¼ cup or 63g	
	White sugar	2 teaspoons heaped or 15g	
	Sasko Dumpy brown bread	1 slice, medium thickness, machine sliced or 30g	
	Stork margarine, tub	Medium spread or 15g	
	Fine apricot jam	Thickly spread or 15g	
	Tea	1 cup or 250g	
	2% milk	6 table spoons or ¼ cup or 65g	
	White sugar	2 level teaspoons or 10g	
1 pm	White bread roll	1 round, medium size or 30g	
	Margarine, stork, tub	Medium spread or 15g	
	Tomato slices	4 thin slices, medium tomato or 100g	
	Polony	2 large slices, machine sliced or 30g	
	Coca-cola	1 can, 340ml	
3 pm	Granny smith apple	2 small or 100g	
7 pm	Cabbage stew: cabbage, beef, potato, onion fried in oil, water added	1 ½ cup or 375g	
	White rice, boiled	2 cups or 500g	
	Mixed vegetables, boiled	½ cup or 125g	
	Tea	1 cup or 250ml	
	2% milk	6 table spoons or ¼ cup or 65g	
	White sugar	2 level teaspoons or 10g	
9 pm	Boiled sweets, sparkles	2	

Training record:

1. Please complete the training record, indicate all training activities on the day of keeping the record
2. It is essential that you exercise as you normally do, don't start exercising just because you are keeping a record. Please **do not record your typical daily activities** such as cleaning, climbing the stairs or walking to class. Only record exercise (e.g. a run, spinning class, aerobics, tennis etc.) you do for your sport (if you are an athlete) or exercise you do to keep fit.
3. When filling in the form, please consider the following points and refer to the example on the next page:
 - Specify the type of exercise (e.g. running, cycling etc.)
 - Write down the duration of exercise in minutes
 - Rate how hard you perceive your workout (see below).

RPE (Rating of Perceived Exertion)

 - 1 = very easy
 - 2 = easy
 - 3 = moderate
 - 4 = hard
 - 5 = very hard

Supplements:

1. Please state clearly the brand and the name of the supplement used
2. Indicate how many times a day you take the supplement and how much of the supplement you take at a time (see the example)

Example:

Brand name:	Type of supplement	Dose (ml/ number of pills)	When did you consume the supplement	How long have you been taking this supplement?
E.g. Vitargo	Recovery drink / Carbohydrate supplement	One scoop (50g) with 500ml water	Immediately after training	> 2 months

Training Record

Please specify the types of exercise and duration performed today:

Types of training:	Duration of exercise:	Distance covered:	Rating of perceived exertion:
Swimming			
Cycling			
Running			
Gym / Weights			
Other, please specify:			

Supplements consumed:

Please indicate the type and amount of supplements you consumed today:

Brand name:	Type of supplement	Dose (ml/ number of pills)	When did you consume the supplement	How long have you been taking this supplement?
E.g. Vitargo	Recovery drink / Carbohydrate supplement	One scoop (50g) with 500ml water	Immediately after training	> 2 months

Food Record, Day 2: Saturday 7 May 2011[illegible]

Training Record

Please specify the types of exercise and duration performed today:

Types of training:	Duration of exercise:	Distance covered:	Rating of perceived exertion:
Swimming			
Cycling			
Running			
Gym / Weights			
Other, please specify:			

Supplements consumed:

Please indicate the type and amount of supplements you consumed today:

Brand name:	Type of supplement	Dose (ml/ number of pills)	When did you consume the supplement	How long have you been taking this supplement?
E.g. Vitargo	Recovery drink / Carbohydrate supplement	One scoop (50g) with 500ml water	Immediately after training	> 2 months

Food Record, Day 3: Sunday 8 May 2011 (Race day)

[illegible]

Training Record

Please specify the types of exercise and duration performed today:

Types of training:	Duration of exercise:	Distance covered:	Rating of perceived exertion:
Swimming			
Cycling			
Running			
Gym / Weights			
Other, please specify:			

Supplements consumed:

Please indicate the type and amount of supplements you consumed today:

Brand name:	Type of supplement	Dose (ml/number of pills)	When did you consume the supplement	How long have you been taking this supplement?
E.g. Vitargo	Recovery drink / Carbohydrate supplement	One scoop (50g) with 500ml water	Immediately after training	> 2 months

APPENDIX 3.15 CAFFEINE WITHDRAWAL SYMPTOMS QUESTIONNAIRE

Subject number: _____

During the time that you excluded caffeine intake (24 April – 6 June 2011), did you experience any of the following symptoms:

☐ Headaches

☐ Fatigue

☐ Lethargy

☐ Flu-like symptoms

If you answered yes to any of the above questions, please specify when and how severe the symptoms were:

APPENDIX 3.16 CAFFEINE SIDE EFFECTS QUESTIONNAIRE

Subject number: _____

Did you experience any of the following symptoms before, during or after triathlon 1 and triathlon 2:

☐ Nervousness

☐ Shakiness

☐ Anxiety

☐ Heart palpitations

☐ Flushing

☐ Gastro-intestinal disturbances like vomiting and or diarrhoea

☐ Sleep alterations

☐ Headaches

If you answered yes to any of the above questions, please specify when and how severe the symptoms were:

APPENDIX 3.17 LETTER OF ETHICS APPROVAL



UNIVERSITEIT-STELLENBOSCH-UNIVERSITY
jou kennisvenoot • your knowledge partner

02 November 2010

MAILED

Mrs S. Potgieter
Division Human Nutrition
Department of Interdisciplinary Health Sciences
P.O. Box 19063
Tygerberg
7500
Fax: +27 21 933 2991

Dear Mrs Potgieter

PROTOCOL: NONE

"The effect of caffeine supplementation on Olympic distance triathletes and triathlon performance in the Western Cape region"

ETHICS REFERENCE NO: M10/08/030

RE : APPROVED

At a meeting of the Health Research Ethics Committee that was held on 1 September 2010, the above project was approved on condition that further information is submitted.

This information was supplied and the project was finally approved on 1 November 2010 for a period of one year from this date. This project is therefore now registered and you can proceed with the work.

Please quote the above-mentioned project number in ALL future correspondence.

Please note that a progress report (obtainable on the website of our Division: www.sun.ac.za/rds) should be submitted to the Committee before the year has expired. The Committee will then consider the continuation of the project for a further year (if necessary). Annually a number of projects may be selected randomly and subjected to an external audit. Translations of the consent document in the languages applicable to the study participants should be submitted.

Federal Wide Assurance Number: 00001372

Institutional Review Board (IRB) Number: IRB0005239

The Health Research Ethics Committee complies with the SA National Health Act No. 61 2003 as it pertains to health research and the United States Code of Federal Regulations Title 45 Part 46. This committee abides by the ethical norms and principles for research, established by the Declaration of Helsinki, the South African Medical Research Council Guidelines as well as the Guidelines for Ethical Research: Principles Structures and Processes 2004 (Department of Health).

Please note that for research at a primary or secondary healthcare facility permission must still be obtained from the relevant authorities (Western Cape Department of Health and/or City Health) to conduct the research as stated in the protocol. Contact persons are Ms Claudette Abrahams at Western Cape Department of Health (healthres@pgwc.gov.za; Tel: +27 21 483 9907) and Dr Hélène Visser at City Health (Helene.Visser@capetown.gov.za; Tel: +27 21 400 3981). Research that will be conducted at any tertiary academic institution requires approval from the relevant hospital manager. Ethics approval is required BEFORE approval can be obtained from these health authorities.

02 November 2010 15:58

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Fakulteit Gesondheidswetenskappe · Faculty of Health Sciences



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Afdeling Navorsingsontwikkeling en -steun · Division of Research Development and Support
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Tel.: +27 21 938 9075 · Faks/Fax: +27 21 931 3352



UNIVERSITEIT-STELLENBOSCH-UNIVERSITY
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Approval Date: 1 November 2010

Expiry Date: 1 November 2011

Yours faithfully

MS CARLI SAGER

OFFICE FOR CLINICAL TRIALS

Tel: +27 21 938 9140 / E-mail: carli@sun.ac.za

Fax: +27 21 931 3352

02 November 2010 15:58

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UNIVERSITEIT STELLENBOSCH UNIVERSITY
jou kennisentrum • your knowledge partner

04 May 2011

MAILED

Mrs S Potgieter
Division Human Nutrition
Department of Interdisciplinary Health Sciences
P O Box 19063
Tygerberg
7500
Fax: +27 21 933 2991

Dear Mrs Potgieter

PROTOCOL: NONE

"The effect of caffeine supplementation on Olympic distance triathletes and triathlon performance in the Western Cape region"

ETHICS REFERENCE NO: M10/08/030

RE : PROTOCOL AMENDMENT

Your letter dated 17 March 2011 refers.

The chairperson of the Health Research Ethics Committee approved the amendment to the protocol on 19 April 2011.

The ratification of the scientific merit and feasibility of the amendment should be confirmed by the Faculty Committee for Postgraduate Education.

Yours faithfully

MR FRANKLIN WEBER

OFFICE FOR CLINICAL TRIALS

Tel: +27 (0)21 938-9657 / E-mail: fweb@sun.ac.za

Fax: +27 (0)21 931-3352

04 May 2011 10:10

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APPENDIX 3.18 PILOT STUDY INFORMED CONSENT FORM

<p align="center">Informed Consent form for participation in the PILOT study</p> <p>Subject Reference number: _____</p>
--

PARTICIPANT INFORMATION LEAFLET AND CONSENT FORM

TITLE OF THE RESEARCH PROJECT:

The effect of caffeine supplementation on Olympic distance triathletes and triathlon performance in the Western Cape region

REFERENCE NUMBER: M10/08/030

PRINCIPAL INVESTIGATOR: Sunita Potgieter, Stellenbosch University

ADDRESS:

Department of Interdisciplinary Health Sciences
Division Human Nutrition
Stellenbosch University and Tygerberg Academic Hospital
PO Box 19063
Tygerberg
7505
South Africa

CONTACT NUMBER: +27 21 938 9474 or +27 82 335 3650

You are being invited to take part in a research project. Please take some time to read the information presented here, which will explain the details of this project. Please ask the study staff or doctor any questions about any part of this project that you do not fully understand. It is very important that you are fully satisfied that you clearly understand what this research entails and how you could be involved. Also, your participation is **entirely voluntary** and you are free to decline to participate. If you say no, this will not affect you negatively in any way whatsoever. You are also free to withdraw from the study at any point, even if you do agree to take part.

This study has been approved by the Health Research Ethics Committee (HREC) at Stellenbosch University and will be conducted according to the ethical guidelines and principles of the international Declaration of Helsinki, South African Guidelines for Good Clinical Practice and the Medical Research Council (MRC) Ethical Guidelines for Research.

What is this research study all about?

- The study will be conducted at a gym in the Helderberg region, Western Cape Province.
- The main aim of the study is to test certain questionnaires as well as to test if we can collect a blood sample during the transition from the cycle to the run during a triathlon.
- The researcher will host one sprint triathlon, consisting of a 750 m swim, 20 km cycle and 5 km run.

The following is what will happen during the pilot study:

Before the triathlon you will have a meeting with the main researcher. The researcher will ask you to sign the informed consent form and explain all the details of what the study entails.

The researcher will ask you to sign the informed written consent form (if not yet signed), as well as the indemnity waiver. During this meeting you will also complete the following questionnaires:

- Demographic questionnaire
- Medical history questionnaire
- Training regime questionnaire
- Habitual caffeine intake questionnaire

You will also have your height and weight measurements taken by the researcher.

On the morning of the race, a registered nurse will place a saline lock in your arm. This will allow the nurse to draw blood during various time points in the triathlon. A blood sample will be taken before the start of the race, during the transition from the cycle to the run and after the run leg by a registered nurse.

You will also be asked to complete the Borg scale rating of perceived exertion during the transition 1 (swim to cycle), transition 2 (cycle to run) and at the finish line. The researcher will explain this scale to you before the triathlon.

Why have you been invited to participate?

You have been invited to participate in this study because you are a triathlete residing in the Western Cape region.

What will your responsibilities be?

Your main responsibilities will be to participate in the triathlon, to complete the necessary questionnaires and to consent to the blood samples which will be taken.

Will you benefit from taking part in this research?

All triathletes will benefit from taking part in this project. This pilot study forms part of a bigger study to determine whether or not caffeine supplementation can improve exercise performance.

Are there risks involved in your taking part in this research?

- The risks involved in taking part in this study include the risk of participating in a triathlon. It is advised that you make sure you are physically fit in order to complete the triathlon.
- There will be a minimal risk involved when inserting the saline lock catheter into your arm for drawing the blood samples. The risk involves slight bruising or bleeding under the skin as a result of the venipuncture, which is the procedure used to draw blood. When the catheter (saline lock) is inserted, it will be covered with Tegaderm to ensure water does not enter at the site of the catheter. We will also instruct you to be careful when taking off their wetsuits, to ensure you do not pull out the saline lock catheter. The STATLOCK system we will be using is a needle free system and poses no risk beyond that of the venipuncture when inserting the catheter.

If you do not agree to take part, what alternatives do you have?

Participation in this study is completely voluntary.

Who will have access to your medical records?

All the information collected will be completely confidential and protected. To ensure anonymity, each subject will receive a number and names will not be used. Contact details of the participants will be destroyed directly after the data collection period.

What will happen in the unlikely event of some form injury occurring as a direct result of your taking part in this research study?

Medical assistance and life guards will be available at the triathlon to assist with any medical problems. It is advised that you are physically fit to complete a sprint distance triathlon before agreeing to partake in this research project.

Will you be paid to take part in this study and are there any costs involved?

No, you will not be paid to take part in the study.

Is there anything else that you should know or do?

- You should inform your family practitioner or usual doctor that you are taking part in a research study and ensure that you are physically and medically fit to complete a sprint distance triathlon.
- You should also inform your medical insurance company that you are participating in a research study.
- You can contact Sunita Potgieter at telephone: +27 82 335 3650 or +27 21 938 9474 if you have any further queries or encounter any problems.
- You can contact the **Health Research Ethics Committee** at 021-938 9207 if you have any concerns or complaints that have not been adequately addressed by your study organizer.
- You will receive a copy of this information and consent form for your own records.

Declaration by participant

By signing below, I agree to take part in a research study entitled "Caffeine supplementation influences Olympic distance triathletes and triathlon performance in the Western Cape region"

I declare that:

- I have read or had read to me this information and consent form and it is written in a language with which I am fluent and comfortable.
- I have had a chance to ask questions and all my questions have been adequately answered.
- I understand that taking part in this study is **voluntary** and I have not been pressurised to take part.
- I may choose to leave the study at any time and will not be penalised or prejudiced in any way.
- I may be asked to leave the study before it has finished, if the study doctor or researcher feels it is in my best interests, or if I do not follow the study plan, as agreed to.

Signed at (place) on (date) 2009.

Signature of participant

Signature of witness

Declaration by investigator

I Sunita Potgieter declare that:

- I explained the information in this document to
- I encouraged him/her to ask questions and took adequate time to answer them.
- I am satisfied that he/she adequately understands all aspects of the research, as discussed above
- I did/did not use a interpreter. (If an interpreter is used then the interpreter must sign the declaration below.

Signed at (place) Stellenbosch on (date) 2009.

Signature of investigator

Signature of witness

Declaration by interpreter

I (*name*) declare that:

- I assisted the investigator (*name*) to explain the information in this document to (*name of participant*) using the language medium of Afrikaans/Xhosa.
- We encouraged him/her to ask questions and took adequate time to answer them.
- I conveyed a factually correct version of what was related to me.
- I am satisfied that the participant fully understands the content of this informed consent document and has had all his/her question satisfactorily answered.

Signed at (*place*) on (*date*)

Signature of interpreter

Signature of witness

APPENDIX 3.19 RESEARCH STUDY INFORMED CONSENT FORM

Informed Consent Form
Subject Reference number: _____

PARTICIPANT INFORMATION LEAFLET AND CONSENT FORM

TITLE OF THE RESEARCH PROJECT:

The effect of caffeine supplementation on South African Olympic distance triathletes and triathlon performance

REFERENCE NUMBER:

PRINCIPAL INVESTIGATOR: Sunita Potgieter, Stellenbosch University

ADDRESS:
 Department of Interdisciplinary Health Sciences
 Division Human Nutrition
 Stellenbosch University and Tygerberg Academic Hospital
 PO Box 19063
 Tygerberg
 7505
 South Africa

CONTACT NUMBER: +27 21 938 9474 or +27 82 335 3650

You are being invited to take part in a research project. Part of the research project also involves genetic analysis. Please take some time to read the information presented here, which will explain the details of this project. Please ask the study staff or doctor any questions about any part of this project that you do not fully understand. It is very important that you are fully satisfied that you clearly understand what this research entails and how you could be involved. Also, your participation is **entirely voluntary** and you are free to decline to participate. If you say no, this will not affect you negatively in any way whatsoever. You are also free to withdraw from the study at any point, even if you do agree to take part. This study has been approved by the Health Research Ethics Committee (HREC) at Stellenbosch University and will be conducted according to the ethical guidelines and principles of the international Declaration of Helsinki, South African Guidelines for Good Clinical Practice and the Medical Research Council (MRC) Ethical Guidelines for Research.

What is Genetic research?
 Genetic material, also called DNA or RNA, is usually obtained from a small saliva sample. Genes are found in every cell in the human body. Our genes determine what we look like, what kind of diseases we may be susceptible to and sometimes how we metabolize different nutrients. Worldwide, researchers in the field of genetics are continuously discovering new information that may be of great benefit to future generations and also that may benefit people today.

What is this research study all about?

- The study will be conducted in the Helderberg region, Western Cape Province. The researcher will invite all the triathletes in the Western Cape to participate in the research study.
- The main aim of the study is to determine whether caffeine supplementation influences Olympic distance triathletes and therefore their triathlon performance. The researcher would like to determine whether caffeine supplementation has a significant beneficial effect on exercise performance, rate of perceived exertion and the body's response to stress.

- The genetic component of the study involves measuring the prevalence of CYP1A2 gene polymorphism in your body. This will give us insight as to how your body metabolizes or breaks down caffeine. We would like to see if there are differences in the way the different athletes break down caffeine, to determine who will benefit most from caffeine supplementation before a triathlon.
- The researcher will host two triathlons, two weeks apart. You will be asked to participate in both triathlons.
- During the first triathlon a randomly selected sample will receive a capsule containing a tasteless white powder with 6mg/kg body weight caffeine and the other group will receive a capsule containing only a tasteless white powder. Neither the participants nor the researcher will know who receives the caffeine. During the second triathlon, the group who received the caffeine supplementation in the first triathlon, will now receive placebo and the group who received the placebo, will now receive 6mg/kg body weight caffeine supplementation. The amount of caffeine given to you, does not pose any adverse health risks to you.

The following is what will happen during the research study:

Prior to enrolment:

Before the first triathlon you will have a meeting with the principle investigator. The researcher will ask you to sign the informed consent form and explain all the details of what the study entails.

Time point 1: Three days before triathlon 1 (Pre-registration)

The researcher will ask you to sign the informed written consent form (if not yet signed), as well as the indemnity waiver. During this meeting you will also complete the following questionnaires:

- Demographic questionnaire
- Medical history questionnaire
- Training regime questionnaire
- Habitual caffeine intake questionnaire
- Profile of Mood States questionnaire

You will also have your height and weight measurements taken by the researcher. The researcher will accompany you to have your body composition analyzed with a DEXA scan at Stellenbosch University. The DEXA scan will give you and the researcher the results of your body composition, including your bone mineral density, fat mass and fat free mass.

The researcher will explain in detail what the study entails and what your involvement in the study will be. The researcher will give you a small scale and the instructions on how to complete a food record. You will be asked to write down exactly what you eat, and weigh the portions on the scale provided on the two days before each race, as well as on race day. This will give the researcher an idea of your nutritional preparation before a race, as well as your race day nutrition strategies.

You will start to keep the food record on the Friday, Saturday and Sunday (race day). A saliva sample will also be taken from you to determine how you metabolize caffeine (CYP1A2).

We will provide you with all the pre-race information.

Time point 2: On the day of Triathlon 1 (3 hours before the start of the race)

On the morning of the race, a registered nurse will take a blood sample from your arm. One blood sample will be taken 3 hours before the start of the race by a registered nurse. This blood sample will be analysed for plasma caffeine, full blood count, serum cortisol, prolactin, testosterone, DHEAs and serum albumin. These parameters will give the researcher and

idea of your stress response. A volunteer will also prick your finger to measure capillary lactate levels.

You will be asked to complete the Profile of Mood States questionnaire again and another weight measurement will be taken.

Race briefing for triathlon 1 will take place.

Time point 3: On the day of Triathlon 1 (1 hour before the start of the race)

You will be given either 6 mg/kg body weight caffeine OR the placebo capsule. Both the caffeine and the placebo capsules contain a white tasteless powder. Neither the researcher nor the subjects will know who is receiving the caffeine and who is receiving the placebo.

Time point 4: On the day of Triathlon 1 (Start of the race)

The race will start with a 1.5 km swim. Thereafter you will enter transition 1 where you will complete the Borg scale rating of perceived exertion and start the 40 km bike route. During the transition from the bike to the 10 km run, you will complete the Borg scale rating of perceived exertion again and another blood sample will be taken by a registered nurse. This will be analyzed for plasma caffeine and serum albumin. The volunteer will prick your finger for another capillary lactate sample. We will record the time it takes the nurse to draw the blood sample and subtract that from your total time to complete the triathlon. Then you will commence the 10 km run leg of the triathlon. At the finish line, another blood sample will be taken by a registered nurse and analyzed for plasma caffeine, full blood count, serum cortisol, prolactin, testosterone, DHEAs and serum albumin. A volunteer will prick your finger 5 times (3,6,9,12 and 15 minutes after the race) to measure capillary lactate. These parameters will give the researcher an idea of your stress response. You will also be asked to complete the Borg scale rating of perceived exertion as well as the Profile of Mood States questionnaire again. You will also have time to complete the food record for the rest of the day.

Time point 5: Three days before triathlon 2:

You will be asked to write down exactly what you eat, and weigh the portions on the scale provided on the two days before triathlon 2, as well as on race day (food record). This will give the researcher an idea of your nutritional preparation before a race, as well as your race day nutrition strategies.

You will start to keep the food record on the Friday, Saturday and Sunday (race day). We will provide you with all the pre-race information for triathlon 2.

Time point 6: On the day of Triathlon 2 (3 hours before the start of the race)

The researcher will ask you to sign the indemnity waiver for triathlon 2. During this meeting you will also complete the following questionnaire:

- Profile of Mood States questionnaire

On the morning of the race, a registered nurse will take a blood sample from your arm. One blood sample will be taken 3 hours before the start of the race by a registered nurse and analyzed for plasma caffeine, full blood count, serum cortisol, prolactin, testosterone, DHEAs and serum albumin. These parameters will give the researcher an idea of your stress response. A volunteer will also prick your finger for a capillary lactate sample.

Race briefing for triathlon 2 will take place.

Time point 7: On the day of Triathlon 2 (1 hour before the start of the race)

You will be given either 6 mg/kg body weight caffeine OR the placebo capsule. You will receive the opposite one you received during triathlon 1. Both the caffeine and the placebo capsules contain a white tasteless powder. Neither the researcher nor the subjects will know who is receiving the caffeine and who is receiving the placebo.

Time point 8: On the day of Triathlon 2 (Start of the race)

The race will start with a 1.5 km swim. Thereafter you will enter transition 1 where you will complete the Borg scale rating of perceived exertion and start the 40 km bike route. During

transition 2 you will complete the Borg scale rating of perceived exertion again and another blood sample will be taken by a registered nurse and analyzed for plasma caffeine and serum albumin. A volunteer will prick your finger to measure capillary lactate levels. The time needed to take the blood sample will be recorded and subtracted from your total time to complete triathlon 2. Then you will commence the 10 km run leg of the triathlon. At the finish line, another blood sample will be taken by a registered nurse and analyzed for plasma caffeine, full blood count, serum cortisol, prolactin, testosterone, DHEAs and serum albumin. These parameters will give the researcher an idea of your stress response. A volunteer will prick your finger 5 times (3,6,9,12 and 15 minutes after the race) to measure capillary lactate. You will also be asked to complete the Borg scale rating of perceived exertion as well as the Profile of Mood states questionnaire again. You will also have time to complete the food record and hand it in at the end of the race. After the last athlete has finished the race, a prize giving will be held where the first 5 athletes of each gender and age category will receive their prize money. All the time splits will be recorded.

Why have you been invited to participate?

You have been invited to participate in this study because you are one of 'Western Provinces' elite / amateur triathletes and you have completed an olympic distance triathlon in the year before the study.

What will your responsibilities be?

Your main responsibilities will be to participate in both triathlons, to complete the necessary questionnaires and to consent to the body composition measurements and blood samples which will be taken. It will also be expected of you to complete two food records and weigh the portion sizes of the food you eat.

Will you benefit from taking part in this research?

All triathletes will benefit from taking part in this project. Caffeine is a known ergogenic aid during exercise. The ultimate goal of any athlete is to improve exercise performance. Caffeine has the ability to improve your exercise performance, but research in the field setting (in a race) and research in triathlon specifically is limited.

Are there any risks involved in your taking part in this research?

- The risks involved in taking part in this study include the risk of participating in a triathlon. It is advised that you make sure you are physically fit in order to complete the triathlon.
- There will be a minimal risk involved when we draw the blood samples. The risk involves slight bruising or bleeding under the skin as a result of the venipuncture, which is the procedure used to draw blood.
- Caffeine withdrawal can be expected in subjects who habitually use > 300 mg caffeine per day (more than 3 cups of coffee). Symptoms include headaches, fatigue, lethargy and flu-like symptoms. The researcher will ask you avoid caffeine-containing food and drink for 14 days prior to triathlon 1 and triathlon 2 (24 April – 23 May 2011). This will allow sufficient time for withdrawal symptoms to pass. If any of these symptoms are present, you should contact the researcher immediately and medical care will be made available.
- Since the acute dose of caffeine supplementation is also > 300mg per athlete, there is a risk of acute withdrawal on the day of supplementation. In the case of acute withdrawal after either triathlon 1 or triathlon 2, you will have access to medical care. The acute withdrawal symptoms are the same as listed above (headaches, fatigue, lethargy and flu-like symptoms).
- Although the dose of caffeine is not high enough to cause adverse effects, side effects that may occur include; nervousness, shakiness, anxiety, heart palpitations, flushing, gastro-intestinal disturbances, sleep alteration and headaches. If any of these symptoms are present, you will have direct access to medical care.

If you do not agree to take part, what alternatives do you have?

- Participation in this study is completely voluntary.

- Caffeine supplementation only enhances exercise performance and it is not intended to treat or cure any medical condition.

Who will have access to your medical records?

- All the information collected will be completely confidential and protected. To ensure anonymity, each subject will receive a reference number and names will not be used. Contact details of the participants will be destroyed directly after the data collection period.

What will happen in the unlikely event of some form injury occurring as a direct result of your taking part in this research study?

- Medical assistance and life guards will be available at both the triathlons to assist with any medical problems. It is advised that you are physically fit to complete an Olympic distance triathlon before agreeing to partake in this research project.

Will you be paid to take part in this study and are there any costs involved?

No, you will not be paid to take part in the study but there will be prize money available after the second triathlon. To emulate the race environment, prize money will be given to the first 5 male and female athletes after each triathlon in the respective age groups (20-39 and 40-60 years).

How long will your blood be stored and where will it be stored?

All your blood samples will be sent to Pathcare for analysis, except for the blood sample collected to determine the full blood count. This sample will be analyzed by a laboratory that is part of the Department of Physiology of Stellenbosch University. The saliva sample will be analyzed for CYP1A2 gene polymorphism. This sample will be taken to the Department of Genetics, Stellenbosch University for analysis.

If your blood / saliva is to be stored is there a chance that it will be used for other research?

Your blood / saliva will only be used for genetic research that is directly related to the CYP1A2 gene polymorphism (the way you metabolize caffeine). Also if the researchers wish to use your stored blood for additional research in this field they will be required to apply for permission to do so from the Human Research Ethics Committee at Stellenbosch University. If you do not wish your blood specimen to be stored after this research study is completed you will have an opportunity to request that it be discarded when you sign the consent form.

How will your confidentiality be protected?

All the information collected will be completely confidential and protected. To ensure anonymity, each subject will receive a reference number and names will not be used. Contact details of the participants will be destroyed directly after the data collection period.

Is there anything else that you should know or do?

- You should inform your family practitioner or usual doctor that you are taking part in a research study and ensure that you are physically and medically fit to complete an Olympic distance triathlon.
- You can contact Sunita Potgieter at telephone: +27 82 335 3650 or +27 21 938 9474 if you have any further queries or encounter any problems.
- You can contact the **Health Research Ethics Committee** at 021-938 9207 if you have any concerns or complaints that have not been adequately addressed by your study organizer.
- You will receive a copy of this information and consent form for your own records.

Declaration by participant

By signing below, I agree to take part in a research study entitled "The effect of caffeine supplementation on South African Olympic distance triathletes and triathlon performance"

I declare that:

- I have read or had read to me this information and consent form and it is written in a language with which I am fluent and comfortable.
- I have had a chance to ask questions and all my questions have been adequately answered.
- I understand that taking part in this study is **voluntary** and I have not been pressurised to take part.
- I may choose to leave the study at any time and will not be penalised or prejudiced in any way.
- I may be asked to leave the study before it has finished, if the study doctor or researcher feels it is in my best interests, or if I do not follow the study plan, as agreed to.

Tick the option you choose:

☐ I agree that my blood or tissue sample can be stored indefinitely/ stored for ... years, but I can choose to request at any time that my stored sample be destroyed. My sample will be identified with a special study code that will remain linked to my name and contact details. I have the right to receive confirmation that my request has been carried out. (NB This option can be excluded completely if the genetic research has no clinical relevance to the patient and you plan to completely and permanently anonymise all samples)

OR

☐ I agree that my blood or tissue sample can be stored **indefinitely/ stored for,years** after the project is completed but that it is anonymised with all possible links to my identity removed, and that the researchers may then use it for additional research in this or a related field. Once my sample is anonymised, my rights to that sample are waived. My sample may be shipped to another laboratory in SA or abroad to be used in other research projects in this or a related field

OR

☐ Please destroy my blood sample as soon as the current research project has been completed.

Signed at (place) on (date) 2009.

.....
Signature of participant

.....
Signature of witness

Declaration by investigator

I Sunita Potgieter declare that:

- I explained the information in this document to
- I encouraged him/her to ask questions and took adequate time to answer them.
- I am satisfied that he/she adequately understands all aspects of the research, as discussed above
- I did/did not use a interpreter. (If an interpreter is used then the interpreter must sign the declaration below.

Signed at (*place*) Stellenbosch on (*date*) 2009.

Signature of investigator

Signature of witness

Declaration by interpreter

I (*name*) declare that:

- I assisted the investigator (*name*) to explain the information in this document to (*name of participant*) using the language medium of Afrikaans/Xhosa.
- We encouraged him/her to ask questions and took adequate time to answer them.
- I conveyed a factually correct version of what was related to me.
- I am satisfied that the participant fully understands the content of this informed consent document and has had all his/her question satisfactorily answered.

Signed at (*place*) on (*date*)

Signature of interpreter

Signature of witness

APPENDIX 3.20 INDEMNITY WAIVER TO COMPETE IN THE TRIATHLON(S)

Indemnity form				
Subject Reference number: _____				
<p>I, the undersigned,</p> <p>_____, hereby agree</p> <p>to participate in two triathlons as part of a research study.</p> <p>I confirm that my participation in the event and the related activities is entirely voluntary and I accept all risks involved therein. The researcher or any of the organizing committee or Stellenbosch University and or any of their respective employees or partners shall not be liable for any loss, damage, injury or illness of whatsoever nature and howsoever caused, suffered by me (to my person or property) as a result, directly or indirectly, of participating in the two events.</p> <p>Signed at: _____ on this _____ of _____ 2010</p> <table style="width: 100%;"><tr><td style="width: 50%; border-top: 1px solid black; text-align: center;">Signature of participant:</td><td style="width: 50%; border-top: 1px solid black; text-align: center;">Print Name:</td></tr></table> <table style="width: 100%;"><tr><td style="width: 50%; border-top: 1px solid black; text-align: center;">Signature Witness:</td><td style="width: 50%; border-top: 1px solid black; text-align: center;">Print Name:</td></tr></table>	Signature of participant:	Print Name:	Signature Witness:	Print Name:
Signature of participant:	Print Name:			
Signature Witness:	Print Name:			

APPENDIX 3.21 RANDOMIZED CONTROLLED CLINICAL TRIAL INSURANCE



Alexander Forbes

RISK SERVICES

20 May 2010

TO WHOM IT MAY CONCERN

STELLENBOSCH UNIVERSITY: CONFIRMATION OF INSURANCE ON CLINICAL TRIALS

Title of the project

"Caffeine supplementation influences olympic distance triathlon performance"

This serves to confirm that the following cover has been arranged for clinical trials in terms of the following two policies:

1. No fault Compensation Insurance policy number SPRGL0900457 underwritten by Lloyds for a limit of USD5 000 000.
2. Professional Liability Insurance policy number P01380 underwritten by Stalker Hutchison Admiral for a limit of ZAR132 000 000. This policy has been extended to include Medical Malpractice.
3. Period of insurance: 1 January 2010 to 31 December 2010.

Subject to the terms, conditions and exclusions of the policy.

We trust that you will find the above to be in order. Please do not hesitate to contact the writer should you have any queries

Yours sincerely

Joubert Ferreira
Direct Line : 021 809 5548



Alexander Forbes Risk Services (Pty) Ltd
Co. Reg. No : 2007/015286/07
An Authorised Financial Services Provider - FSB/FSP Licence No : 9256

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For your convenience, please refer to our direct lines
Website: www.alexanderforbes.co.za

Directors: MS Moko (Chairman) H Madungundza (Managing Director) GW Bishop * JJ Ennos GS Jameson * BL McCluskey * B Oosthuizen * AJ Oost * S Tshabalala GJ Wintam MH Zimvula ** (* Alternate) (** Independent)

APPENDIX 3.22 FEEDBACK FROM ATHLETES AND PHOTOS OF T1 AND T2

Event: PhD Research Triathlon #1

Date: 22 May 11

Format: Olympic Distance Triathlon – 1.5 Swim, 40 Km Bike and 10 Km Run

Athlete Name: David Sullivan

Race Time: 2:22:44

Position: 11th

On Sunday, the weather decided to take a break between cold fronts and allow us to race, two weeks later than scheduled. I think Mother Nature was given a double dose of caffeine on 8 May and dished out ferocious winds resulting in the event being postponed. Effectively, this meant that all of us triathletes had been caffeine-free for 4 weeks, barring the one off day in-between when we were allowed to indulge in coffee and chocolate.

We are caffeine-free because we are participating in a study to test the effects of caffeine on Olympic distance triathlon performance. This involves taking either caffeine or a placebo pre-race and then giving numerous blood samples as well as filling out mood charts and rating your perceived rate of exertion while racing. Sunita Potgieter is using this study to complete her PhD in Nutrition.

So the wetsuits are on, the ocean is flat and the icebergs have started melting - allowing us a plenty of room to attack what seems to be a 4km swim (even though I am sure the distance on a Olympic Triathlon is 1.5km?). I think that maybe the lifeguard's GPS batteries need changing!

Race briefing is complete and 30 shivering bodies enter the water for a wet start. I had planned to resemble a tuna gliding through the water but instead resembled a can of tuna doing everything in its power to make it back to land!

The bike leg was split into 4 laps of 10 Kms up and down the beach road from Gordons Bay towards Strand and back. Except for the odd car pulling out from the left, which got the heart pumping, everything was organised to perfection and it was an enjoyable ride. I was proudly wearing my skimpy ATC tri kit for the very first time which meant it took 2 laps for me to warm up. The cold did not seem to affect the others.

At one point I saw the purple flash of the speed camera go off but without any cars around... I am sure Viv Williams (1st lady – 3rd overall) must have triggered it because she was flying!

Transition from bike to run included a visit to the medical tent for a lactate finger prick test as well as 2 ampoules of blood taken from the arm. I am sure this wasn't everyone's cup of caffeine-free tea but I welcomed the break to catch my breath.

The run leg consisted of 3 laps of 3.33 Kms along the beach front path towards Bikini Beach and back. It was here that we got to see all of our competitors' faces. Some were smiling, some were grimacing, some were race-faced and some had tears in their eyes (OK, so it was only me) when they got to see how fast the eventual winner, Bradley Weiss, managed to run after swimming and riding!

Once across the finish line, the vampires were at us again for blood. More blood from the arm (another 4 ampoules) as well as a finger pricks every 3 minutes for 15mins. I am now able to sift flour through my fingers...

We now have to get through another 2 weeks, without caffeine, for the second triathlon. Everything will be exactly the same except those who received caffeine before the first triathlon will now receive the placebo and vice versa.

Let's hope Mother Nature gets another placebo so she can be as kind as she was on Sunday...



2011-05-22 Research Triathlon I - Overall.pdf [189.17 kb ~ 1.05 minutes @ 56kbps]

2011-05-22 1st Research TRI









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